RELATIONSHIP BETWEEN TEMPERATURE AND DEVELOPMENT OF THE ECTOPARASITOID *LARRA BICOLOR* (HYMENOPTERA: SPHECIDAE) AND THE ENDOPARASITOID *ORMIA DEPLETA* (DIPTERA: TACHINIDAE)

By

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The rates of development of the second introduced strain (from Bolivia) of the ectoparasitoid wasp *Larra bicolor* and of the two introduced strains (from subtropical Brazil and from temperate Brazil) of the larviparous endoparasitoid tachinid *Ormia depleta* were determined when reared at both constant and field temperatures in northern Florida. The data were used to examine the performance of the accumulated degree-day model (DD) to predict field development of both natural enemies parasitizing the mole cricket *Scapteriscus borellii*. Development and survivorship were studied in incubation chambers at eight constant temperatures, 15.0, 17.5, 20.5, 22.5, 25.0, 27.5, 30.0 and 32.5 ± 0.1°C, at 14:10 (L: D) photoperiod. The relationship between temperature and developmental rate was estimated with linear regression analyses. Pooled developmental times for males and females were used to fit the linear regression models. The lower developmental threshold was estimated by the "x-intercept" method. Development of all stages of the wasp occurred at temperatures from 25.0 to 30.0°C. No development was

observed at 17.5°C, and only one wasp developed at 22.5°C. Development of *O. depleta* occurred in the range from 17.0 to 30.0°C. The field development was determined by confining newly parasitized hosts into 15 × 5 cm (L × diameter) chambers, filled with autoclaved sand, buried underground at up to 60 cm depth that were operated during all parts of the year except the coldest months. Field experiments with *L. bicolor* were run during autumn. Temperature underground was recorded every 30 min during field experiments. A degree-day model based upon constant temperature was fitted to data from field experiments for each parasitoid species and strain. A full description is presented of the developmental time (and rates) in day units, for constant and field experiments, together with the regression coefficients from constant temperatures and the validation of both data sets using the accumulated degree-day model. Data about the wasp are summarized in life tables and its field efficacy in parasitizing mole crickets is presented and explained. Data about the fly comparing development per sex and per strain are presented also.

CHAPTER 1
INTRODUCTION

Three mole cricket species from South America arrived as immigrants to the southeastern USA in ships' ballast about the year 1900 (Walker and Nickle 1981). They are *Scapteriscus abbreviatus* Scudder, *S. vicinus* Scudder and *S. borellii* Giglio-Tos. The immigrant mole crickets became the most important pests of turf and pasture grasses in Florida. Their feeding and tunneling also destroy seedlings of vegetables, ornamentals, and tobacco. Insecticides commonly applied to control mole crickets in lawns, golf courses, and seedbeds are expensive and not always effective. In pastures there is no control that is economically feasible (Walker 1985a).

Adult *S. abbreviatus* have very short wings, and are flightless. Its current distribution is restricted to coastal areas and two small areas inland of Florida. Adult *S. vicinus* and *S. borellii* have wings that slightly exceed the length of the body, and can fly. Their current dispersion includes eight states, from North Carolina south to Florida and west to Texas (Frank and Parkman 1999). In 1978, the University of Florida established a mole cricket research program (MC program) because of damage to pasture grasses (Walker 1985a). Damage (including control costs) to turf and pastures in Florida alone may approach $100 million annually (H. Frank personal communication 2001).

Three biological control agents from South America have been successfully released into Florida for control of immigrant mole crickets, an ectoparasitic wasp *Larra bicolor* F., an endoparasitic fly *Ormia depleta* Wiedemann, and a parasitic nematode *Steinernema scapterisci* Nguyen and Smart (Frank and Parkman 1999).

The neotropical digger wasp *Larra bicolor* (Hymenoptera: Sphecidae) has been known for decades as a parasitoid of large nymphs and adults of mole crickets (Castner 1984). A Puerto Rican stock originally from Brazil was introduced into Florida in 1981. *L. bicolor* wasps were released at five sites in south and central Florida in conjunction with plots of their preferred nectar-source plant *Spermacoce verticillata L.*

Establishment of *Larra bicolor* only at Ft. Lauderdale, and not at the four more northerly sites in Florida, suggested that this wasp, of tropical origin, could not withstand colder or longer winters farther north in Florida (Castner 1988). In the late 1980s a different, possibly more cold-tolerant strain of *L. bicolor* was imported from Bolivia, released in northern Florida, and a decade later appeared not only to be established but also to be spreading, indicating at least that mole crickets in some unknown proportions were being parasitized (Frank et al. 1995).

Ormia depleta is an endoparasitoid of *Scapteriscus* spp. adults. Females find hosts by homing on the sounds of calling male crickets (Fowler 1987). *O. depleta* was imported from Piracicaba, Brazil (23°30'S), released at numerous sites in Florida, and soon spread throughout south and central Florida. This first strain failed to establish permanent populations in north Florida (>27° N), perhaps due to its poor tolerance to cold and long winters (Walker et al. 1996). In recent years, in late autumn, a few parasitic flies have been captured near Gainesville, and these were considered to be individuals dispersing from south toward north without re-establishing populations. In 1999 a second tachinid strain was imported from southern Brazil (30ºS); because of its origin farther south (in a cooler climate) it was hoped that this strain might be more cold-tolerant and might establish populations north of 29ºN in Florida and other states (Frank 1994).

The entomopathogenic nematode *Steinernema scapterisci* Nguyen and Smart (Rhabditida) was imported from Uruguay and released in Florida in 1985 (Parkman and Frank 1992). This nematode successfully attacks adults and large nymphs of *S. borellii* and *S. vicinus*, but is not very effective against *S. abbreviatus*, and has negligible effect against small and medium nymphs of any of these three pest mole crickets (Frank and Parkman 1999).

There is no information about the effect of temperature on life history or seasonal phenology of any of the insect parasitoids. Limits of geographical expansion of *L. bicolor* and *O. depleta* are poorly understood. The fact that the first introduced strains failed to establish in northern Florida suggests a strong influence of temperature regimes, where either colder winters kill the parasitoids or cause the lack of adequate energy (nectar, etc.) sources for the adults. These arguments led to the introduction of a second strain with the hope that they would be more cold hardy and survive colder winters.

The level of control of mole crickets exerted by these two insect parasitoids in Florida is questionable (e.g., Potter 1998). The questions arise from lack of adequate data.

Development of plants and invertebrates is temperature-dependent with each organism requiring a specific measure of heat accumulation between lower and upper developmental thresholds to complete development (Hagstrum and Milliken 1988). Application of developmental thresholds and rates in the form of phenological models is often used in agricultural IPM programs to predict and manipulate pest and natural enemies population dynamics in the field (Tauber et al. 1994).

Temperature plays a critical role in determining the rate of development, survival, and reproduction of insect species. Understanding the relationship between temperature

and various life-history processes is important in developing and refining computer models for simulating biological control of pest populations. Physiological time measured in degree-days is effective in predicting the seasonality of life history stages of insects. The development of a degree-day model requires the determination of the temperature threshold above which development will occur. Developmental thresholds can be determined directly in the laboratory by measuring the time taken to rear insects through a series of developmental stages at a range of constant temperatures. Then the thresholds can be validated in the field (Wagner et al. 1984a, Campbell et al. 1974).

Phenology of many species of poikilotherms as a function of temperature has been described by regression equations (Wagner et al. 1984b). Such regression equations can provide a method for calculating developmental times in insect population models or can be used for quantitative comparisons of the effect of temperature on the developmental times of different stages and species of insects (Hagstrum and Milliken 1988).

Understanding the effects of temperature on summary life-history parameters such as the intrinsic rate of increase can be useful for examining the potential effectiveness of a species as a biological control agent, estimating its potential for population growth (Lysyk 2000).

Information describing the life history of pest mole crickets and of their two insect natural enemies released in Florida is inadequate. In the best of the cases, the phenology of the parasitoid is based upon development at a single temperature. Thus, we lack the information necessary to describe development and reproduction of any of the

insects involved. No development model has been proposed for any of the imported insect natural enemies of mole crickets.

The objective of this study was to investigate the development of the digger wasp *Larra bicolor* and of the tachinid fly *Ormia depleta* parasitising mole crickets at both constant and field temperatures. These data are used to examine the feasibility of using a degree-day accumulation model to predict the development of the two parasitoids in the field.

This dissertation is organized by parasitoid species. The studies on the parasitic wasp present the development of the wasp at constant temperatures followed by the field study and finally a degree-day model simulating the wasp development in the field and its validation.

The parasitic fly studies are composed of three experiments; firstly, studying its development at constant temperatures, then in field experiments and finally presenting the proposal of a degree-day model and its validation in field development of this parasitoid.

CHAPTER 2
REVIEW OF LITERATURE

Introduction

Three species of mole cricket (*Scapteriscus* spp.) are the most damaging pests of turf and pasture grasses in much of the southeastern United States. They destroy turf and grasses by feeding on roots, leaves and shoots, and by tunneling, which disrupts the soil around roots, preventing uptake of water and nutrients (Hudson 1997, cited in Frank and Parkman 1999, Walker 1985a). Damage to Florida turf was estimated as $30 million annually in Florida alone (Walker 1985a), $45 million (damage and cost of control) in 1986, with a further $33 million in Alabama, Georgia, and South Carolina combined (not including damage in North Carolina, Mississippi, Louisiana, and Texas) (Frank and Parkman 1999). More recent estimates are of annual expenditure of more than $18 million on chemical insecticides in Florida turf, and of more than $12 million in damage in Georgia turf (Hudson et al. 1988). Damage to pasture grass in Georgia in 1996 was estimated as 0.83 million for control and as $9.8 million in damage (Bunti

n et al. 1997, cited in Frank and Parkman 1999).

The most promising strategy for solving immigrant mole cricket problems is the use of classical biological control. In 1978, in response to increasing concerns of cattleman, turf managers, and homeowners, the University of Florida initiated a research project on mole crickets (Walker 1985a). Three biological control agents from South America have been successfully released into Florida for control of adventive mole

crickets, *Larra bicolor* F. (Hymenoptera: Sphecidae), *Ormia depleta* Wiedemann (Diptera: Tachinidae) and *Steinernema scapterisci* Nguyen and Smart (Rhabditida: Steinernematidae) (Frank and Parkman 1999).

Information regarding natural history of the *Scapteriscus* mole crickets and of the insect parasitoids in the continental United States is presented in this chapter.

The Pest Mole Crickets, *Scapteriscus* spp.

Four mole crickets (Orthoptera: Gryllotalpidae) are listed as occurring in Florida: *Neocurtilla hexadactyla* Perty, the northern mole cricket; *Scapteriscus abbreviatus* Scudder, the shortwinged mole cricket; *Scapteriscus borellii* Giglio-Tos, the southern mole cricket; and *Scapteriscus vicinus* Scudder, the tawny mole cricket. The northern mole cricket is the only species native to the southeastern USA including Florida. The three other mole cricket species are immigrants from South America that were accidentally brought in by ships' ballast about the year 1900 (Walker and Nickle 1981).

Mole crickets are fossorial insects with greatly enlarged forelegs that are used for digging. Some of the adults and nymphs of a population move about on the soil surface on warm nights with high humidity. Depth of the mole crickets in the soil depends upon temperature and moisture (Frank and Parkman 1999, Lake 2000). High water tables force the mole crickets to the surface. Males are more active than females; maximum average depth reported by Abate (1979) was 50 cm for *S. borellii* males and 44 cm for *S. vicinus* males. *Scapteriscus* mole cricket burrows as deep as 75 cm have been recorded (Frank and Parkman 1999). Subterranean depths of oviposition by *S. borellii* and *S. vicinus* are

30 cm maximum depth recorded in the field by Hayslip (1943) and a maximum mean depth of 60 cm for *S. borellii* from laboratory experiments by Lake (2000).

Adults of *S. abbreviatus* have very short wings. In contrast, wings of *S. borellii* and *S. vicinus* slightly exceed the length of the body, which explains their current wide distribution in the southern coastal plains from North Carolina south to Florida and west to Texas. *S. abbreviatus* cannot fly and its distribution is restricted to some small areas in Florida (Frank and Parkman 1999). *S. borellii* and *S. vicinus* sometimes fly in enormous numbers. Their flights apparently serve two functions: (1) local searching for mates and new egg-laying or calling sites and (2) long range dispersal (Walker 1985b).

Adult mole crickets, like many other crickets, produce a calling song that functions to bring the sexes together. Females sometimes respond to the call by walking or flying to the male (phonotaxis) and mating with him. The calling period is correlated with female activity and flight periods (Walker 1985b). The intensity of the song depends upon male size and soil moisture (Forrest 1985).

The following information was presented by Walker (1985b). Mole crickets spend nearly all their lives underground. Adult *S. vicinus* fly in large numbers typically in March but as early as February in Florida after warm winters. They mate (although some matting occurred the previous autumn); they begin as eggs laid in underground chambers. Eggs take some three weeks to incubate. Nymphs hatch from eggs as early as April, but continue to hatch from later deposited eggs through June. Nymphs take some five months to develop during summer months, and newly formed adults begin to appear in September. There is a minor peak of flight activity in the autumn, as early as August in the far south and as late as December farther north if the weather remains warm. In most

of the southern USA, the spring activities occur in *S. borellii* about three weeks later than in *S. vicinus*, but the autumnal activities are at the same time. From central Florida northward, *S. vicinus* and *S. borellii* have one generation each year. Most *S. vicinus* overwinter as adults, and most *S. borellii* overwinter as large juveniles. In southern Florida *S. vicinus* maintains its one year life cycle, but *S. borellii* has two generations annually. In total contrast, all developmental stages of *S. abbreviatus* occur throughout the year but with two peak ovipositional periods, one in late spring and one in winter.

The Digger Wasp *Larra bicolor*

Natural History of *Larra bicolor*

Wasps of the genus *Larra* (Hymenoptera: Sphecidae) are the only sphecids known to attack mole crickets, which all of them do. Unlike other digger wasps, which are generally predators, all *Larra* wasp species are parasitoids (Sailer 1985). Worldwide, 65 species of *Larra* are known (Bohart and Menke 1976). Only one species, *Larra analis* F., a parasitoid of the northern mole cricket, is native to the United States. Sixteen species have been recorded from South America, where the diversity of species of mole crickets belonging to the genus *Scapteriscus* is greatest (Sailer 1985). The following information was presented by Sailer (1985). Apart from preferences for different species of mole crickets, the biology of the various species of *Larra* is remarkably similar. Females do not construct a nest, and prey paralysis is temporary with the host reviving soon after egg deposition. A single egg is firmly attached in the soft, membranous thoracic tissue between the host's first and second legs. The *Larra* larva lives ectoparasitically until almost fully grown. It then kills and consumes the rest of its host. *Larra* females lay about 30 eggs during their life span. Full-grown larvae form underground cocoons of

sand grain cemented together with a secretion from the labial glands. The *L. bicolor* adults emerge from their cells 60 to 80 days after eggs are laid on the hosts. At Fort Lauderdale (south Florida) at least four generations are probably produced annually. Females hunt for mole crickets during early morning and mid afternoon hours. During the middle of the day they visit flowers where they feed on nectar and possibly pollen. *L. bicolor* is most commonly found on the flowers of two quite unrelated plants, *Spermacoce verticillata* L. (Rubiaceae) (native to south Florida) and *Hyptis atrorubens* L. (Lamiaceae). The close association of *Larra bicolor* with these plants suggests that it may not inhabit areas where these plants are not available.

Life Cycle of *Larra bicolor* in the Laboratory

The following information was presented by Castner (1988). The following data were recorded from parasitoids reared in a laboratory maintained at approximately 26 ± 2ºC and 65 ± 5% relative humidity. The 4.0-4.5 mm *Larra* egg hatches in 6-7 d. The first instar larva remains fixed to the original point of attachment. The larva passes through five instars, feeding initially on hemolymph, but progressing to body tissues as heavily sclerotized mandibles develop. The larva completes the first four instars 8-9 d after hatching, increasing gradually in size to 12-19 mm. The final larval size is 14-26 mm. By 10-11 d post-hatching (16-18 d post-oviposition), the wasp grub has killed the host and consumed the soft inner tissues. The mature larva spends approximately one day in constructing an ovate cocoon of soil particles or sand grains. The wasp adult emerges 6-8 weeks after the cocoon is completed.

Establishment of *Larra bicolor* in Florida

The wasp *Larra bicolor* was the first classical biological control agent of mole crickets to be introduced and established in Florida. A Puerto Rican stock originally from Brazil was introduced in 1981; wasps were released at five sites in south and central Florida in conjunction with plots of their preferred nectar-source plant *Spermacoce verticillata*. Establishment of *Larra bicolor* only at Ft. Lauderdale, and not at the four more northerly sites in Florida, suggested that this wasp, of tropical origin, could not withstand colder or longer winters farther north in Florida (Castner 1988, Frank and Parkman 1999). In 1988-89 a Bolivian stock of *L. bicolor*, possibly more cold-tolerant, was introduced, and a decade later appeared not only to be established but also to be spreading in northern Florida indicating at least that mole crickets in some unknown proportions were being parasitized (Frank and Parkman 1999).

The Tachinid Fly *Ormia depleta*

Natural History of *Ormia depleta*

All species of the family Tachinidae develop as parasites of other arthropods. Some groups of species are restricted to closely related kinds of host insects; for example the group Ormiini consists of species that parasitize katydids and other night-singing Orthoptera (Sailer 1985). *Ormia depleta* is a South American tachinid that attacks mole crickets of the genus *Scapteriscus* (Fowler 1987). Adults are crepuscular and nocturnal parasitoids. Females are phonotactic, locating their hosts by their calling songs, and they deposit larvae on or near the host (Fowler and Kochalka 1985).

The first stock was brought from Piracicaba, Brazil (23°S), and released in Florida in 1988. Since then it has become established in 38 counties in Florida, and was

considered as largely responsible for a decline in mole cricket populations (Parkman et al. 1996). No evidence of their extension farther to the north exists despite releases in northwestern Florida, Georgia, Alabama, and North Carolina (Frank et al. 1996, Walker et al. 1996). By 1994, this strain disappeared from the Gainesville area (29° 40'N latitude), perhaps due to its poor tolerance to cold and long winters (Walker et al. 1996). In recent years, in late autumn, a few parasitic flies have been captured near Gainesville, and these were considered to be individuals dispersing from south toward north without re-establishing populations.

In 1999 a second tachinid strain was imported. It was hoped that this "new" strain, from 30°S in Brazil, might be more cold-tolerant and could establish populations north of 29°N in Florida and other states (Frank 1994, Potter 1998).

Several studies were performed with the first strain of *Ormia depleta*, focused on establishment and range (Frank et al. 1996), seasonality (Walker et al. 1996), effectiveness in controlling mole crickets (Parkman et al. 1996), techniques for rearing in the laboratory (Wineriter and Walker 1990) and food source of the adults (Welch 2000). This "old" strain was in culture from 1987 (Frank et al. 1996) until 1999, but it is the "new" strain that is now in culture to supply populations.

Life History of *Ormia depleta* in the Laboratory

Wineriter and Walker (1990) determined the life history of the first strain under laboratory conditions at 23-25°C. The life cycle *of O. depleta*, from oocyte to adult, is about 36 d. Numbers of planidia/female at time of examination range from 70 to 310, mean = 187 ± 45, in laboratory-reared females. Most gravid females contain eggs and immature larvae as well as planidia, suggesting that females deposit larvae over many

days. Emergence of adults occurs very early in the morning and spans a 3-4 d period. Emergence of males generally precedes that of females. Sex ratio is usually 1:1.

Insect Modeling

Numerous independent variables influence arthropod development but none is more important than temperature. In fact, the effects of temperature are so profound that most phenology models consider only this one variable. Other biotic and abiotic variables may influence development times, such as food quantity and quality, population density, humidity, rainfall, pH, and photoperiod (Hagstrum and Milliken 1988, Wagner et al. 1991). Population factors can also influence development times, for example genetic differences (Wagner et al. 1991) and gene expression (Gilbert and Raworth 1996).

The temperature dependence of insect development has been frequently investigated. Temperature affects both the time of development as well as fecundity; consequently, the appearance and dynamics of insect populations in the field are often dictated by ambient temperatures. For this reason there has been considerable interest for a long time in the temperature relationship of development time and fecundity mainly of filthy, annoying, and disease-transmitting insects in order to apply it in predicting the outbreak-time and the dynamics of pests (Ratte 1985).

Predicting the seasonal occurrence of insects is essential for accurate scheduling of census samples and control tactics. These predictions require an understanding of insect development, and because temperature has a major effect on this process, the thermal requirements of development are often a basic to prediction (Wagner et al. 1984b).

Temperature determines life of organisms more than do any other environmental factors. The cause of this temperature sensitivity lies in the fundamental fact that chemical compounds build all organisms and that all processes of life are made by chemical reactions that follow the laws of thermodynamics. Temperature not only acts upon the rate of all chemical reactions according to the Arrhenius equation, but also causes conformational transitions of proteins, phase transition of lipids, change in the structure of water, etc. (Alexandrov 1977, cited by Hoffman 1985). However, many results from field observations and laboratory experiments in which the insects have been subjected to fluctuating temperatures cannot be brought into this concept. A too simplistic physiological concept of the temperature action might, however, lead to erroneous insight into the insect's capabilities of adapting to various thermal environments (Ratte 1985).

Constant vs Fluctuating Temperature Influence on Insect Development

Generally the velocity of insect development increases with elevation of temperature. Many workers have constructed hyperbolae by plotting the constant temperatures at which the insects were reared against the duration of development (Wigglesworth 1972). Janish (1930), cited in Wagner et al. (1984b), stated that the reciprocal of the hyperbolae represents the relative velocity of development, and the line based on the reciprocal shows the rate of acceleration of development with rising temperature. From these observations numerous empirical and physiochemical formulations of insect development have been made. Two such formulations are the day-degree or temperature summation rule and the non-linear inhibition model (Behrens et al. 1983).

Wagner et al. (1984b) presents a very clear explanation of what thermal summation or rate summation means: many empirical and biophysical models describe the time versus temperature or the rate versus temperature curve of insect development. Models of rate versus development relationships are used widely to predict insect development times because mean daily (or hourly) rates can be accumulated under fluctuating temperature environments. These mean rates are summed to equal 1.0, at which time mean cohort development is complete. However models of time versus temperature relationships are primarily descriptive, although the inverse of these regressions also yields development rates.

Constant Temperatures

Typically the development time of a larva decreases exponentially with increasing temperature. In almost all insects, at lower temperatures the development time decreases steeply with increasing temperature; at medium temperatures the slope of the curve becomes smaller until it reaches zero at the so-called optimal temperature for development, which lies usually close to the upper limit. The curve of developmental rate (reciprocal developmental time) takes a more or less sigmoidal course including an inflexion point mostly between 20° and 30°C, as well as a maximum typically between 25° and 30°C (Ratte 1985). See Figure 1.

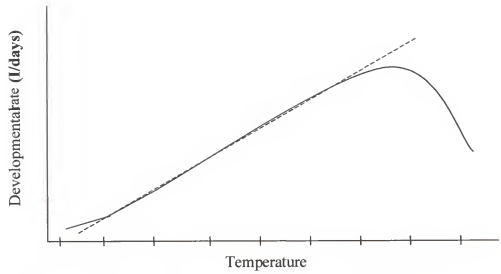

Figure 1. Theoretical linear and nonlinear model development rate functions.

The developmental rate curve displays a wide, approximately linear, range around inflexion point; a straight line often fits this part of the curve. Consequently, the time of development follows a hyperbolic function of temperature over this range. Similar relationships between developmental rate and temperature have been found frequently and have led to establishment of the "rule of thermal summing" and the classical method of "temperature summation" (Ratte 1985).

$TC = t_d (T-T_0)$
Where:
t_d : time of development
TC : thermal constant
T : experimental temperature
T_0 : threshold temperature
$T-T_0$: effective temperature above T_0.

In other words, the time of development multiplied by the effective temperature ($T-T_0$) is a constant (thermal constant). "Thermal summing" implies the existence of a distinct threshold temperature (T_0), at which the developmental rate becomes zero

(developmental zero; developmental threshold). The developmental threshold has to be regarded as theoretical because it can be extrapolated only from a linear rate relation and cannot be determined by rearing experiments; the threshold may fall outside the favorable range for development, and also the infinite developmental time at temperatures near the threshold makes its measurement practically impossible. Although the cessation of development below critical temperatures can be observed easily, the concept of real developmental zero has not been unequivocally accepted. When subjected to sub-threshold temperature some insects develop faster after subsequent exposure to normal temperature indicating that some development also takes place below the threshold (Andrewartha and Birch 1954, Ratte 1985).

Ratte (1985) points to an important phenomenon of developmental rate curves: the degree of temperature dependence, expressed as slope of the rate curve, or Q_{10}, shows a wide variation among various insects and some do indeed possess the capability to develop independent of ambient temperature (exemplified with the carpet beetle, *Attagenus megatoma*).

Fluctuating Temperatures

The developmental time of insects reared under a temperature fluctuating regime has usually been found to differ from that under the corresponding constant temperature. Considerably higher acceleration may occur when the low temperature falls below the developmental threshold. The same has also been observed with nondaily alternating temperatures or single transfers from sub-threshold to favorable temperatures. Consequently, it has been concluded that under thermoperiod conditions also below the threshold "some development may take place": this is assumed to occur because the

temperature requirements of some rate-limiting development steps are nevertheless satisfied (Ratte 1985).

In cases where in one species different thermoperiods have been tested within the entire favorable range for development, the following rule has often been observed: temperature fluctuations in the range below the inflexion point of the developmental rate curve may cause an acceleration in development, while the converse is true for thermoperiods alternating about the temperature optimum; there are slight or no deviations in developmental time for constant temperatures with fluctuations around medial temperatures of the favorable range. This rule was propounded quite early and can also be derived from the non-linearity of the developmental rate versus temperature relation established under constant temperature (Ratte 1985).

Applicability of Constant and Fluctuating Temperatures in Insect Developmental Models

Most of the models, called physiological models, transform temperature into a physiological scale, such as the classical degree-day summations (Wagner et al. 1984b). To describe field stage frequency data- that is number of individuals in each stage of development- in most cases parameters are fitted in two steps. First, parameters of the physiological scale are estimated from constant temperatures in the laboratory. Second, the parameters of the phenological model are estimated from the proportion of individuals in each development stage on the physiological scale. The applicability of the phenological model requires that thermal constants, determined from constant temperatures in the laboratory, can be extrapolated to natural conditions (Wagner et al.

1984b, Candy 1991, Manel and Debouzie 1997a). However, development rates at constant temperatures often differ from those at fluctuating temperatures (Worner 1992).

Manel and Debouzie (1997a) say that deriving the relation from different constant temperatures may be questioned; a model aimed at predicting insect development in natural populations requires other experimental designs - by recording temperature directly in the field or in the laboratory under fluctuating temperatures. Degree-day models extrapolated to natural conditions may or may not be successful for many reasons not related to temperature.

Studies of the effect of fluctuating temperatures on insect development often show that, given the same mean temperature, insects appear to develop at rates that differ under fluctuating conditions from those at constant temperatures. Such studies may indicate that development is accelerated, decelerated, or the same as that under constant conditions. Such variability in reports appears to arise from studies where a limited temperature range is investigated (Worner 1992).

Temperature-Dependent Models for Studies on Insect Development

With the advent of computer simulation to the applied biology sciences, there has been increased interest in mathematical models that describe organism growth and development as a function of temperature (Sharp and DeMichele 1977). Many empirical and biophysical models describe the time versus temperature or the rate versus temperature curve of insect development. Models of rate versus temperature relationships are used widely to predict insect development times because mean daily (or hourly) rates can be accumulated under fluctuating temperature environments (Worner 1992).

Models are important tools for predicting future events for development times in particular and population dynamics in general (Ellsbury 1991). Many pest management

systems rely on models of arthropod and host phenology, and there seems to be no end to the assemblage of new approaches contrived for this purpose (Wagner et al. 1991).

Statistical and deterministic simulation modeling rely on a complex process made of trials, errors, and gradual improvement of the simulations (Mesplé et al. 1996) consider the major problem is to be able to quantify the quality of the simulations in order to know whether a modification of the concepts, the laws simulating the processes or the parameters improve it.

Linear Model

This model assumes a linear relationship between development rate and constant temperature; therefore, the number of degree-days (or thermal units) required to complete development is assumed constant at all favorable temperatures.

The duration of development is calculated by adding up the number of thermal units (degree-hours or degree-days) contributed at each temperature (Wagner et al. 1984b cite very old reports: Candolle 1855 and Reibisch 1902 among others). The thermal constant or degree-days for completed development are most easily derived from the reciprocal of the slope of the regression line fitted to development rate data, obtained typically at constant temperatures (Worner 1992). This method has been applied to plant phenology (Schroder and Sondgerath 1996)

This degree-day approach is used widely because it requires minimal data for formulation, is easy to calculate and apply, and often yields approximately correct values. However this approach is valid only over intermediate temperatures. Furthermore, the threshold temperature is often determined empirically by extrapolating the straight line to the temperature axis. For these reasons, the number of degree-days required for complete

development is too low at temperatures near the lower threshold and too high at or above the optimum. These limitations are important and should not be overlooked when interpreting the results of this technique (Wagner et al. 1984b, Manel and Debouzie 1997a, b).

The rate summation formula is

$$D = \sum r[T(t)]\Delta t$$

where the proportions of development taking place at each time increment are summed so that, when D equals 1, the insect has completed development.

This model measured in physiological units is not purely descriptive or statistical in nature but is biologically motivated. However it is based on population attributes rather than the progression of individuals through stages over time (Candy 1991).

In spite of inherent limitations of the thermal model, to that linear portion of insects' developmental rate and the obligated definition of a lower threshold, this thermal model cannot be ruled out. As expressed by Liu and Meng (1999) insect development may occur most frequently at some temperature range considered in that degree-day summation and the model may fit well with natural varying temperature development although the logistic model better estimates development below the lower threshold. Logan et al. (1976) considered the degree-day model invalid for mite populations. Tanigoshi et al. (1976) showed that this model did not estimate development of field populations of mites.

Gilbert and Raworth (1996) said that in insects, development rates increase almost linearly with temperature over the normal range of operating temperatures. The published data cover more than 300 species, and in every case the effect of temperature on

development rate is effectively linear, but falls off at average daily temperatures higher than those normally experienced in the field. Lamb (1992) working with aphid clones detected some genuine curvature at low temperatures, but for practical purposes the relation was linear.

Belehradek (1935) (cited in Andewartha and Birch 1954) described the response curve with an exponential equation that becomes linear on a log scale. This approach does an inadequate job of describing development rates at the temperature extremes.

Non-linear Models

Because of the difficulties of predicting insect development at temperature extremes, empirical non-linear models have been proposed, attempting to explore this response beyond the intermediate curve temperatures. Non-linearity of development (the Kauffman effect, Worner 1992) or rate summing effect (Ratte 1995) implies that development rates at constant temperature differ from those at fluctuating temperatures. Models have been developed to incorporate nonlinearity and variable temperatures (Hagstrum and Milliken 1991, Manel and Debouzie 1997a, b).

Examples of nonlinear models are presented below (some are described in Table 2-1):

The symmetric and asymmetric catenary model. Proposed by Janish (1930) and cited by Wagner et al. (1984b), this model describes development time as a function of temperature. This equation combines two exponential curves, the reciprocal of which describes the accelerating phase of development rate up to the optimal temperature and the decelerating phase beyond the optimum. This model is criticized for inadequate fits and computational difficulties.

Second- and third-degree polynomials. These have been used to regress development time against temperature although they have not been used for prediction purposes (Tanigoshi et al. 1976). Hagstrum and Milliken (1988) compared the polynomial model: $d = t + t^2$ in which d is developmental time, and t is centigrade temperature, and was less accurate than the Sharp and DeMichele (1977) model in describing developmental time of nine species of stored-product Coleoptera under various temperatures, moisture levels, and diets.

Logistic equation. This describes development rates as a function of temperature (Davidson 1944). This equation is used widely although it is not very descriptive at one or both ends of the response curve. Manel and Debouzie (1997a) concluded that this model gives relatively close estimates of stage duration when applied to a single stage of development and when base temperature is known; but, when base temperature is misestimated, stage duration is either under- or overestimated). Liu and Meng (1999) demonstrated that the logistic model better fit field varying temperature, especially when temperature falls below the lower developmental threshold. This model gives better estimates than the Wang nonlinear model (Wang et al. 1982).

An exponential equation. Similarly, a normal distribution model is proposed by Pradhan (1945 and 1946 as cited in Wagner et al. 1984b). When compared with the logistic model, it yields better results (Siddiqui and Barlow 1972).

A modified sigmoid equation model. This model was proposed by Stinner et al. (1975) to describe the effects of temperature on development rates. This model does not work well at higher temperatures.

The two exponential equations model. Logan et al. (1976) described developmental rates at intermediate and high temperatures. Logan's model was more descriptive than Stinner's modified sigmoid equation and third degree polynomial (Logan et al. 1976). Lactin et al. (1995) modified Logan's model; it fitted better at lower temperatures in ten of eleven cases. They considered this model better than Sharp and DeMichele's model. Ryoo et al. (1991) considered this model appropriate in describing development of a larval parasitoid. Gould and Elkinton (1990) compared the linear model, Logan's model, and Sharp and DeMichele's model, in studying the development of a wasp parasitoid, Logan's model better estimated development at temperatures below 15 °C. No differences were observed at range temperatures of 15° and 30°C.

A complex biophysical model. This model proposed by Sharpe and DeMichele (1977) describes the non-linear response in development rates at both high and low temperatures as well as the linear response at intermediate temperatures. Schoolfield et al. (1981) improved this model making it suitable to the nonlinear approaches. This model is considered as the most suitable to describing insect development rates as a function of temperature by Wagner et al. (1984b). Shaffer (1983) applies this model to 194 cases from the literature, failing in 20% in explaining variations in developmental rate, making this model not true for all species. Hagstrum and Milliken (1991) say this model provides an alternative to degree-day model accumulation method for prediction of developmental times at fluctuating temperatures in the field.

Stochastic Models

Insects reared under identical conditions develop at different rates due to inherent differences among individuals in a population. This stochastic aspect of development is

important, although usually overlooked, in population models of poikilotherm development. It is not surprising, therefore, to find many models, linear and nonlinear, that describe the effect of temperature on insect development; however, most of these models only predict development time from mean rate versus constant temperature relationships. Usually, no consideration is given to the variability in insect development time among individuals. On the other hand, models that consider the stochastic aspects of development time use frequency distributions to determine the probabilities of development time for individuals in a population (Wagner et al. 1984b, Candy 1991, Manel and Debouzie 1997b).

Wagner et al. (1984 a) say that three general stochastic approaches are recognized:

Monte Carlo techniques use pseudorandom numbers to estimate the development time of individuals in a sample population (Menke 1973, Hardman 1976). The disadvantages of this approach are that it is computationally time-consuming because random numbers must be generated for each individual in the same population, and its outputs must be interpreted statistically. Phelps et al. (1993) consider this method as feasible because it uses a fixed number of individuals from one generation to the next and simulates the timing of events rather than the population dynamics of the insects.

Stinner et al. (1975) developed a cumulative function that is fitted to the developmental times at <1, 25, 50, 100% of measured distributions. This probability distribution provides a good description of distributions at different constant temperatures, but when used in population models, the function is not fitted directly to measured distributions.

The final stochastic approach mentioned by Wagner et al. (1984b) applies a probability function directly to the measured distributions. This approach has a mechanistic basis proposed by Sharp et al. (1977). These investigators used normal and quadratic probability density functions to describe the variability in development rates. Although these functions often provide good results, their goodness-of-fit capabilities are unsatisfactory when distributions of rate are asymmetric.

Wang model. Described by Wang et al. (1982), this gives a very good fit to data over a whole range of constant temperatures that permit complete development. Liu and

Meng (1999) compared this model with some other nonlinear models, such as Sharpe and DeMichele (1977), Stinner et al. (1975) and Logan et al. (1976), and found that Wang model offered the highest R^2 and the least estimation bias.

In order to try to minimize the effect of asymmetry of distributions of rate, Wagner et al. (1985) propose the Weibull function to describe the distributions of normalized development time with good results. Ellsbury (1991) applies this same function on development of immature stages of clover stem borers at constant temperatures with good results. The predicted distribution of development time can be used to optimize census sampling and control procedures, or it can be used as input into larger population dynamics models (Wagner et al. 1985).

Continuation ratio model. This model deals with probability of individuals being in each stage, as proposed by Candy (1991) and re-evaluated by Manel and Debouzie (1997a, b). This model applied to stage (multistage) frequencies obtained by random daily sampling, gives unbiased estimates only when a common physiological scale applies to each stage. When stages differ in physiological scale it gives non-independent estimates of duration. The Weibull distribution is integrated to this model. It gives a better fit than the logistic model.

Mixed Models

Recent phenological models incorporate temperature-dependent development and inherent stochastic variation. This approach estimates the proportion of insects in a given development stage as a function of accumulated heat or degree-days (Dennis and Kemp 1988)

Stage successive logistic model. Manel and Debouzie (1997a) combined rate summation, logistic equation and a stochastic description of individuals in a binomial probability. This model gives a better estimate than the continuation ratio model (Candy 1991).

Conclusion

As a way to express something that reflects what I perceived through the writing of this paper, I have chosen to quote what Wagner et al. (1985) conclude: 'The introductory paragraph of Uvarov's (1931) classic monograph, Insects and Climate, states "Economic entomologists at the present day are no longer satisfied with merely recording the outbreaks of insect pests and with devising means for their control. They realize more and more that their chief aim and highest ambition must be to foresee and to prevent outbreaks. In order to do this, all conditions accompanying and causing outbreaks must be thoroughly investigated and elucidated; in other words the epidemiology of insect pests must be the central problem of economic entomological research, which should be carried out from the ecological point of view."

There is a greater opportunity today to predict population events, a requisite to prevent outbreaks. Vast amounts of biological and economic data are available on most major pests; and new approaches to population research and modeling, as well as inexpensive high speed computers, provide the means to identify, integrate and apply this knowledge for predictive purposes.

Recent advances in entomology have occurred as a result of interdisciplinary research. Specialists from outside of entomology have, and will continue, to apply new techniques to our field. These techniques must be integrated into the mainstream of entomological application.

Table 1. Insect models cited in the Review of Literature chapter.

Sharpe and DeMichele (1977), modified by Schoolfield et al. (1981):

$$r(T) = \frac{RHO25 \frac{T}{298.15} \exp\left[\frac{HA}{R}\left(\frac{1}{298.15} - \frac{1}{T}\right)\right]}{1 + \exp\left[\frac{HL}{R}\left(\frac{1}{TL} - \frac{1}{T}\right)\right] + \exp\left[\frac{HH}{R}\left(\frac{1}{TH} - \frac{1}{T}\right)\right]}$$

Where:
r(T) =mean development rate at temperature T(°K)
R =the universal gas constant (1.987 cal degree^{-1} mole^{-1})
RHO25= development rate at 25 °C (298.15 °K) assuming no enzyme inactivation
HA =enthalpy of activation of the reaction that is catalyzed by a rate-controlling enzyme.
TL = Kelvin temperature at which the rate-controlling enzyme is half active and half low-temperature inactive.
HL = change in enthalpy associated with low temperature inactivation of the enzyme.
TH = Kelvin inactivation at which the rate-controlling enzyme is high active and half high-temperature inactive.
HH = change in enthalpy associated with high-temperature inactivation of the enzyme.

Wagner et al. (1984) say they are aware of no other poikiloterm development rate model with greater flexibility, goodness-of-fit capability, or of stronger theoretical foundation. Nevertheless, the use of this model is limited to those with experience in nonlinear parameter estimation. To extend the Sharp and DeMichele model to biologists they present a SAS-computer program for its use.

Weibull function:

$$F(x) = 1 - \exp\left(-\left[(x-\gamma)/\eta\right]^{\beta}\right)$$

Where:
F(x) : the probability of complete development at normalized time x
γ, η and β : are estimated parameters

Logistic equation. Liu and Meng (1999):

$$V(T) = K \Big/ \left(1 + \exp(\alpha - \beta T)\right)$$

Where:
K, α and β : are regression parameters fitted to the data of mean development rates V(T)
K means the upper asymptote of this shallow sigmoid curve.
Table 1. Continues.

Logistic regression equation. Manel and Debouzie (1997a):

$$Pi = \frac{1}{1+\exp(\alpha_0 + \alpha_1 DD_i)}$$

Where:
α_0 and α_1 : are parameters to be estimated
DD_I : the number of degree-days accumulated at time t_i

Continuation ratio model. Candy (1992):

$$\begin{cases} p_i = \dfrac{\exp(b_{0i} + b_{1i DD_i})}{1+\exp(b_{0i} + b_{1i} DD_i)} \\ P = \left[1 - \sum_{k=1}^{i-1} P_k\right] \dfrac{\exp(b_{0i} + b_{1i} DDi)}{1+\exp(b_{0i} + b_{1i} DD_i)} \\ P_r = 1 - \sum_{k=1}^{r-1} P_k \end{cases}$$

Where :
b_0 and b_1 : are parameters to be estimated.
DD_I : the number of degree-days accumulated at time t_i

Wang model. Wang et al. (1982):

$$V(T) = \frac{H}{1+\exp(-r(T-T_0))}\left(1-\exp\left(\frac{T-T_L}{\delta}\right)\right)\left(1-\exp\left(-\frac{T_H - T}{\delta}\right)\right)$$

Where:
V(T) : is the mean development rate at temperature T(°C)
H : is the value of the upper asymptote of the curve
r : is the exponential increase rate
T_0 : is the optimum temperature for development
T_L and T_H : are, respectively, the minimum and maximum temperature boundaries
: boundary width at the lower and upper temperatures.

CHAPTER 3
DEVELOPMENT OF THE WASP *LARRA BICOLOR* F.

Development and reproduction of the wasp *Larra bicolor* at constant temperatures

Introduction

Scapteriscus mole crickets are major pests of turf and pasture grasses in the southern USA. The three mole cricket species that arrived about 1900 as immigrants have being under classical biological control investigation since 1979 under the University of Florida mole cricket research project (Walker 1985a).

Two introductions of the ectoparasic digger wasp *Larra bicolor* have been made in Florida. Their different success is reported in chapter 2 (pages 10-11). The role of the environmental temperature has been considered the main limitation for *Larra bicolor* populations to establish and to disperse in northern Florida and in other southeastern states.

As a way to enhance the biological control of this parasitoid, it is important to determine its vital statistics that may explain its seasonal phenology. These vital statistics are an important and basic component for understanding the dynamics of a species (Lowry et al. 1992). Fertility life tables are appropriate to study the dynamics of animal populations, especially arthropods, as an intermediate process for estimating parameters related to the population growth potential, also called demographic parameters (Southwood 1978, Maia et al. 2000).

The general biology of the wasp *L. bicolor* has been poorly studied. Castner (1988) reported developmental time of *L bicolor* under a single temperature regime, but failed to report variance.

Insects develop within a species-specific temperature regime. The inverse relationship between developmental rate and temperature results in a sigmoid curve with a straight line at the intermediate temperatures. This curve is a fundamental characteristic of an insect life history (Taylor 1981, Wagner et al. 1984b).

The most widely used approach to study the effect of temperature on developmental rates of poikilothermic organisms is the thermal summation or degree-day (DD) model. This method is one of the oldest and most commonly used for insect modeling and for predicting biological events controlled by temperature accumulation (Wagner et al. 1984a, b, Higley et al. 1986).

The objective of this study was to investigate the effect of temperature on development, survivorship and reproduction of *Larra bicolor*.

Materials and Methods

Development

Placing a female wasp into a 200-ml plastic vial and then introducing the host induced parasitism of hosts (*S. borellii*). Parasitism occurred almost immediately most of the times. Parasitized hosts were placed individually in 200-ml plastic cups filled with wet-sterilized sand.

Development and survivorship were studied in incubation chambers at seven constant temperatures, 17.5, 20.0, 22.5, 25.0, 27.5, 30.0 and 32.5°C, at 14:10 (L:D) photoperiod. Development and survivorship were observed and recorded every six hours

until adults emerged. Initial wasp cohorts consisted of 29, 34, 36, 38, 38, 39, 39 individuals respectively per temperature studied.

The relationship between temperature and developmental rate (=1/days) was estimated by linear regression analyses. Pooled developmental times for males and females were used to fit the linear models. The lower developmental threshold (t), the temperature at which development is negligible, was estimated by the "x-intercept" method. Values of t were obtained as follows: t = -a/b, where a and b are parameters from the regression equations (where x = temperature, and y = developmental rate).

The degree-days required for development were calculated by using the equation DD=y(T-t) where 'y' is the developmental time (days), 'T' is the temperature during development, and 't' is the lower developmental threshold. DD were determined for each individual at all temperatures where development was recorded and then averaged by stage.

Life-Fertility Tables

Reproduction of two generations developing during different seasons of the year was studied.

The summer cohort. This consisted of wasps collected during July. Wasps collected from the field were provided with host mole crickets and their parasitic progeny were kept in the laboratory at 26 ± 0.5ºC, 65% RH and 14:10 (L:D) photoperiod. Emerging adults were confined in cages, grouped by age. Adult wasps were fed with diluted hummingbird nectar and honey. The cages were additionally provided with a blooming nectar-source plant (*Spermacoce verticillata*). Summer wasps were given host mole crickets starting a day after emergence, 5 to 7 times a day until the wasp's death.

Parasitized hosts were kept under the same laboratory conditions as the adults, until wasp stages completed development.

The autumn cohort. This consisted of wasps from parasitized mole crickets that were kept under field conditions until parasitic stages completed development. Emerged wasp adults during October were subjected to tests. Wasp adults were provided with host mole crickets as for summer wasps. Parasitized hosts were confined underground in screened chambers of 30 cm depth until adult emergence.

Reproductive statistics were summarized in the form of age-specific life fertility tables for both cohorts.

Results

Development

Developmental times for all stages were inversely related to temperatures from 25.0 to 30.0°C (Figure 2). The temperature of 32.5°C caused a varied effect on the stages of this parasitoid; the egg stage maintained a similar reduction in development compared with previous temperatures, larva and prepupa stages reached a plateau in development compared with previous temperature (30.0°C), and pupa stage slowed down development as almost four times the previous temperature (Table 2). No development was observed at 17.5 and 20.0°C; only one wasp developed at 22.5°C. The total development ranged from 142.90 to 40.18 days at 32.5 and 30°C, respectively. Apparently, the upper temperature threshold was reached at 32.5°C. Pupal development required from 72.5 to 91% of the total development time. Although the duration of stages varied with temperature, the proportion of total developmental time spent in each stage was consistent regardless of temperature.

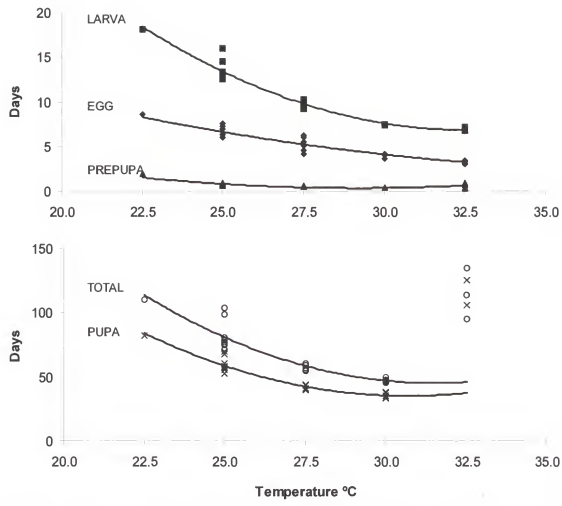

Figure 2. Developmental time of *Larra bicolor* stages and total at constant temperatures.

Table 2. Development (days) of *Larra bicolor* life stages[a] at constant temperatures.

Temp. (°C)	n	Egg	n	Larva	n	Prepupa	n	Pupa	n	Total
32.5	4	3.23 ± 0.09 (2.3)	11	7.33 ± 0.20 (5.4)	4	0.47 ± 0.12 (0.33)	4	130.02 ± 17.38 (91.0)	4	142.90 ± 17.60
30.0	14	3.75 ± 0.28 (8.6)	7	7.38 ± 0.01 (16.6)	8	0.44 ± 0.02 (0.01)	8	35.07 ± 0.69 (75.3)	8	47.50 ± 0.63
27.5	8	4.74 ± 0.63 (9.3)	8	9.87 ± 0.15 (18.4)	6	0.66 ± 0.09 (0.11)	6	41.39 ± 0.80 (72.5)	6	57.06 ± 0.95
25.0	11	5.09 ± 0.28 (6.8)	4	13.30 ± 0.40 (17.2)	9	0.72 ± 0.06 (0.01)	9	62.30 ± 3.76 (75.9)	9	82.10 ± 3.76
22.5	1	8.63 ± 0.00 (7.8)	1	18.13 ± 0.00 (18.2)	1	2.00 ± 0.00 (0.02)	1	81.88 ± 0.00 (74.0)	1	110.63 ± 0.00

[a]Mean ± SEM; percentage of developmental time required for each life stage given in parenthesis.

The survivorship per stage is shown in Figure 3 and described according to Southwood (1978). The maximum survivorship occurred at 30°C followed by 25 and 27.5°C. Survivorship of cohorts reared at 17.5 and 20.0°C followed a type IV curve (100% mortality occurs prior to the adult stage). Cohorts reared at 22.5 and 32.5°C exhibited a type III curve. The remaining cohorts followed a type I curve (mortality acts most heavily on the old individuals).

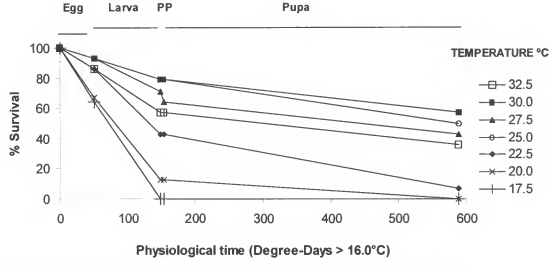

Figure 3. Survivorship of *Larra bicolor* life stages at constant temperatures

Linear regression parameters describing the relationship between developmental rate and temperature for all stages are presented in Table 3. Data for 22.5°C were excluded because only one wasp completed development at this temperature. The data at 32.5°C were not used in the regression model for larva, prepupa and pupa because the values were considered beyond the linear portion of growth responsive to temperature (Sharpe et al. 1977), (Figure 4). Coefficients of determination ranged from 0.71 (prepupa) to 0.94 (larva), indicating a good fit in all cases. At the egg stage, data for 32.5°C were

included, but for all remaining stages data were used for temperatures from 25.0 to 30.0°C, because the upper developmental threshold was apparently reached.

Table 3. Linear regression models (y = developmental rate, x = temperature), lower developmental thresholds (t), and degree-days required for *Larra bicolor* life stages and total to develop at constant temperatures.

Stage	y intercept±SEM	Slope±SEM	R^2±SE	t(°C)	Degree-days (DD±SE)
Egg[a] n = 31	-0.4015±0.0348	0.0221±0.0012	0.92±0.0182	18.143	45.446±0.7107
Larva[b] n = 27	-0.2211±0.0159	0.0119±0.00058	0.94±0.0063	18.620	84.425±0.9331
P-p[b] n = 27	-3.7870±0.7173	0.2032±0.0263	0.71±0.2838	18.640	5.049±0.1652
Pupa[b] n = 27	-0.0389±0.0048	0.0024±0.00018	0.88±0.0019	16.01	413.135±5.642
Total[b] n = 27	-0.03126±0.00276	0.0018±0.0001	0.93±0.001	16.880	540.84±6.035

P-p = prepupa; [a]25.0, 27.5, 30.0 and 32.5°C data only; [b]25.0, 27.7, and 30.0°C data only.

The lower developmental threshold temperature (*t*) obtained by the "x-intercept" method ranged from 16.01 (pupa) to 18.64 (prepupa). In fact, "*t*" values for all single stages were close to 18 except for the pupa, and then for total development. The degree-days (M ± SEM) above their respective "*t*", required by the wasp to develop during different life stages are presented in Table 3. The degree-days for the pupal stage to complete development account for almost 76% of the degree-days required for total development.

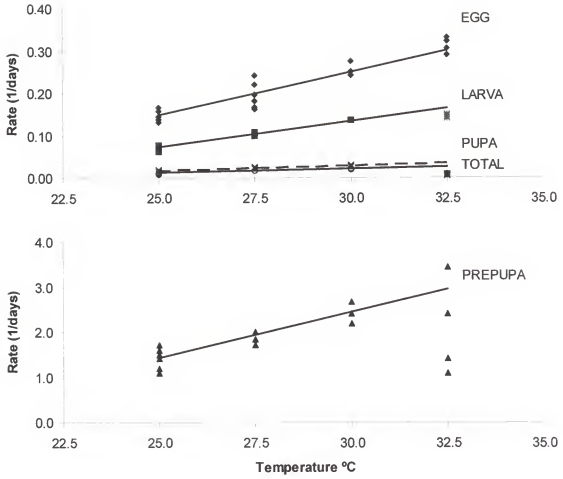

Figure 4. Developmental rates of *Larra bicolor* at constant temperatures. Symbols show observed data. Line trend fitted to the linear response.

Reproduction

Total fecundity (eggs laid per female) was recorded from the two cohorts, summer and autumn wasps (Table 4). Summer wasps oviposited 16 eggs per female, in contrast with 12.5 from autumn wasps. Despite the apparent difference, the means are not statistically different. Egg hatching was statistically the same, with values close to 100% hatching. The female longevity was almost the same in the two cohorts, with a female adult survival of 10 days.

Table 4. Population reproductive statistics for *Larra bicolor* from two seasons in Gainesville, FL (29°40'N).

Season	n females	Fecundity (eggs per female)[a,b]	Fertility (% egg hatch)	Female longevity (days)[a,b]
Summer[c]	7	16.0±2.23[a]	98.2[b]	10.14±1.20[a]
Autumn[d]	4	12.5±2.84[a]	96.4[b]	10.50±3.17[a]

[a]Mean±SEM
[b]Means followed by different letters are significantly different.
[c]Lab-reared wasps at 26 ± 0.5°C.
[d]Field-reared wasps, emerged during October.

Life-FertilityTables

Life fertility tables were constructed for all cohorts to calculate reproductive statistics (Table 5). The net reproductive rate (Ro), which represents the females produced per female per generation of the two populations, overlaps the confidence limits, which makes them statistically not different. The intrinsic rates of increase (Rm) did not overlap each other, meaning that the two populations increase at different rates, with autumn wasps having the slower population growth and being statistically different. The mean generation times (Tc) did not overlap between the two cohorts.

The differences observed in Rm and Tc are explained by the fact that the autumn generation developed under lower temperatures than did the summer generation. These differences are shown by the oviposition periods in Figure 5, where the effect of contrasting temperatures caused delay of oviposition in the autumnal population, and faster oviposition time in the summer population.

Table 5. Reproductive parameters for *Larra bicolor* from two seasons in Gainesville, FL (29°40'N).

Season	Ro[a,d]	Rm[b,d]	Tc (days)[c,d]
Summer[e]	1.76±0.61	0.011±0.006	51.14±5.29
Autumn[f]	1.37±1.00	0.003±0.007	109.47±18.30

[a]Ro = net reproductive rate
[b]Rm = intrinsic rate of increase
[c]Tc = mean generation time
[d]Mean±SEM
[e]Lab-reared wasps at 26 ± 0.5°C.
[f]Field-reared wasps, emerged during October.

Discussion

Development of the digger wasp *Larra bicolor* was characteristic of poikilothermic organisms where developmental time decreases (and developmental rate increases) as temperature increases (Tables 4 and 5, and Figure 4) (Sharpe and DeMichele 1977, Wagner et al. 1984a). The linear developmental rates occurred at temperatures from 25.0 to 30°C for all stages but the egg, where the developmental rate kept its linearity up to the highest temperature of 32.5°C (Figure 4). The upper temperature threshold was passed at 32.5°C for all stages but egg, so that the upper threshold must be at some point between 30 and 32.5°C.

Castner (1988) reared the wasps at 26 ±2 °C and 65% relative humidity, showing a broadest developmental variability for this parasitoid compared with the data reported in the present study where the wasps were reared at temperatures with an accuracy of ± 0.5°C which explains the lower variability in developmental times of the wasp.

The lower threshold value observed in the pupal stage along with the higher number of degree-days required for the pupal stage to develop (almost 76% of the total

development) is a strong indicator that this is the resistant stage to inappropriate environmental temperatures at both extremes (Table 3 and Figure 3). It is important to note that the developmental data presented in this study were obtained from wasps developing during summer and exposed to controlled summer-like conditions. The developmental rates under autumn-like conditions at which this parasitoid is able to develop were not studied, and remain unknown.

L. bicolor developed and survived over a broader temperature range (Table 2) than normally occurs underground during spring to fall seasons in Gainesville. The host mole cricket, *S. borellii*, tolerated extreme temperatures during the study, but the parasitic wasp failed to develop at 17.5 and 20.0ºC, and only one wasp completed development at 22.5ºC. In previous studies (not presented in this dissertation) rearing the three mole cricket species, none survived more than 15 days at temperatures of 15 and 10ºC nor at 35ºC or higher. Those temperature ranges must be outside the limits for these hosts to survive and develop.

Temperature regimes, representing contrasting conditions at which the two cohorts were reared, did not affect reproductive statistics of the parasitoid, considering fecundity, fertility and adult longevity even though the population reproductive parameters estimated from the life-fertility tables (Table 5) showed differences in the intrinsic rate of increase and in generational time, and then in oviposition time (Figure 5). The temperature regime represented by the season of the year did not alter the reproductive capacity of *Larra bicolor*. From these results it can be inferred that the temperature regime (say the season of the year) is not an important constraint of the reproductive performance of this parasitoid, at least in Florida. Other factors such as

suitable hosts and availability of food source for adult wasps should play the more important role restraining the populational increase of *Larra bicolor*.

The developmental time was defined per stage and total life cycle. The lower threshold was determined for all the stages. The upper temperature threshold was reached at 32.5 for all stages but the egg. The degree-day values were estimated for all stages. The reproductive parameters of wasps estimated at summer-like temperature (constant temperatures) were similar to those reared in the field (autumn season).

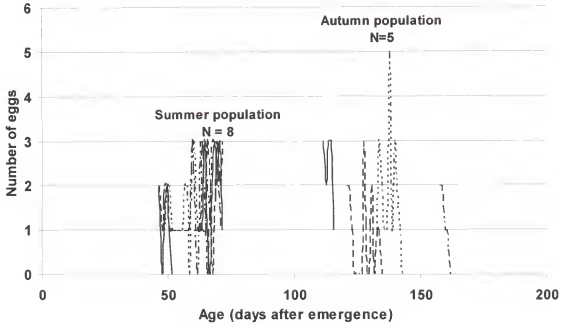

Figure 5. Oviposition time of wasps *Larra bicolor* reared at summer-like and autumnal seasons in Gainesville, FL; daily oviposition per wasp is represented by different line pattern per season.

Development of the wasp *Larra bicolor* at field-varying temperatures.

Introduction.

Studies of the sphecid ectoparasitoid *Larra bicolor* have been devoted to its behavioral ecology and foraging (Fowler 1989), host searching and mate-searching (Castner and Fowler 1987), and establishment and dispersal (Frank et al. 1995). The only information available about the life cycle and field efficiency was obtained by Castner (1988) with the Puerto Rican biotype that established a population in south Florida but failed to do so in central and north Florida. Practically no information about the Bolivian biotype is available. Extensive data are needed to examine the effect on host mole cricket populations, with geographical, seasonal, and annual variation.

Temperature plays a critical role in determining the rate of development, survival and reproduction of host insect species. The duration of development from birth to first reproduction is one of the most important insect life-history characters influencing numerical change in insect populations (Wagner et al. 1984a). Because the rates of development in insects under natural conditions are largely determined by temperature, a realistic measure of the relationship between temperature and development rate is fundamental to any study of the population dynamics or of the management of any insect (Wagner et al. 1984a, Liu and Meng 1999). The degree-day concept is one of the oldest and most widely used entomological models; it assumes a linear relationship between development rate and constant temperatures, therefore the number of degree-days required to complete development is assumed constant at all favorable temperatures (Wagner et al. 1985). Daily temperature cycles that occur in the field are generally not considered by the degree-day method that uses data collected at constant temperatures in the laboratory to predict developmental times in the field (Higley et al. 1986).

Developmental times are known to differ between constant and fluctuating temperatures with the same mean (Hagstrum and Hagstrum 1970). Developmental time data collected at constant temperatures in the laboratory can only be expected to provide a rough estimate of developmental times in the field at fluctuating temperatures (Hagstrum and Milliken 1991).

The relationship of life stages of *Larra bicolor* to constant temperatures is shown in Table 2, resulting in the proposal of a degree-day model (Table 3). The model, based upon constant temperatures, must be evaluated by its goodness of fit to populations developing in the field.

This work describes the development of egg, larval, prepupal, and pupal stages of *Larra bicolor*, Bolivian biotype, under field conditions of Gainesville, FL (29°40' N). The results of this work will be used, in a subsequent study, to evaluate the efficiency of the degree-day model proposed.

Materials and Methods

Female wasps were captured by net from patches of the food-plant *Spermacoce verticillata* L. in Gainesville, FL. After capture, the wasps were placed individually in snap-cap plastic vials, and kept alive by cooling them in a chest. The wasps were taken the same day to the laboratory and placed in groups of 10 wasps into a 30×30×12 cm metal-framed screened cage containing a flowering *S. verticillata*. Water, diluted honey, and diluted artificial hummingbird nectar (®Perky-Pet Products Co.) were supplied also. Unless otherwise indicated the host mole crickets were *Scapteriscus borellii* adults that were captured in traps baited with synthetic mole crickets song at the Ona REC, FL. The hosts were shipped in buckets partly filled with moist vermiculite.

Devices made of PVC pipe were designed to individually confine parasitized hosts underground at a range of depths. These pipe-chambers could be easily buried vertically and are could be easily removed from the ground with little disturbance of mole cricket tunnels inside them. The pipe-chambers were subdivided into four sections with the assumption that each section represented a particular thermal regime and the total individuals confined at each depth can be considered as part of an independent experiment. Thus, it was possible to handle four independent experiments at the same time, covering different possible thermal regimes at which the host mole crickets naturally live and thus the parasitoids develop. The depths of the four chamber sections were: 0-15, 15-30, 30-45, and 45-60 cm. (Table 6).

The pipe-chambers consisted of 5 cm inside diameter × 75 cm long PVC pipes cut lengthwise. Two 2.5 cm wide × 25 cm long windows were cut at the center of each half, covered with aluminum window screen, and sealed with hot melted silicone. The inside of each pipe was subdivided into 15 cm sub-chambers, by placing non-corrosive metallic mesh, fully matching the inner diameter of the pipe once closed, also fixed with hot melted silicone. Starting from the bottom, the pipe-chambers were subdivided into four sub-chambers (15 cm tall × 5 cm diameter), leaving a portion of 15 cm intended to be above the ground level for handling purposes. This chamber design allows the host mole cricket to move freely in a volume of 300 ml of sand. The screened windows allow the sand inside the chambers to be in close contact with the sand outside, allowing the flux of air and moisture from the external soil into the chambers. The temperature was monitored inside and outside the chambers at the different depths, with no difference observed.

Moisture was not measured but was estimated by touch; tap water was added when considered necessary.

Each half chamber was filled with autoclaved moist sand, lightly compressed. Artificial tunnels were made by compressing the moist sand by finger. The halves of the chamber were joined as a sandwich by placing rubber bands at both extremes and at the middle. To check the status of host or parasitoid, or to feed the host, the pipes were withdrawn from the soil, the rubber bands were removed, and each half of the pipe-chamber was rolled gently to a concave position to avoid disturbance of the contents.

Table 6. Codes and depths of sub-chambers and thermocouples where parasitized mole crickets were confined to study the development of *Larra bicolor* underground in the field.

Sub-chambers		Thermocouple
Code	Depth (cm)	Depths (cm)
A	0 - 15	7.5
B	15 – 30	22.5
C	30 – 45	37.5
D	45 - 60	52.5

The land where the pipe-chambers were buried was kept free of weeds for the duration of the experiments. It was then assumed that the irradiation, aeration, and moisture (from environmental humidity and precipitation) were evenly distributed without interference from vegetation. The presence of patches of weeds would have modified the flux of these factors. A rectangle of land, 1m wide × 6m long, located near the Entomology and Nematology Department building, was tilled to 75 cm depth to allow the manipulation of the pipe-chambers in and out of the ground. Before introducing the parasitized hosts, the pipe-chambers were buried underground for a few days. The

chambers were symmetrically distributed, allotting five chambers to the width and 20 chambers to the length of the experimental land. Parasitized hosts were randomly allotted to the sub-chambers.

The soil temperature at which the parasitized host was confined in the sub-chambers was recorded by placing a thermocouple in the middle of the corresponding sub-chamber (Table 6). Temperatures were recorded as follows using one pipe-chamber buried in the center of the experimental land. Four thermocouples were used, one at the depth of each of the four pipe sub-chambers. The thermocouples were connected to a digital temperature datalogger (®HOBO Mod H8-4, Onset Co.) programmed to record temperatures every 30 min. The temperature recorded was downloaded to a computer for further analysis. Another digital temperature datalogger was used to record air temperature, placing the thermocouple at 1.8 m height above ground under a 20×20 cm shed roof.

The parasitism of hosts was achieved following the procedure described by Williams (1928) cited by Castner (1988). Placing a female wasp into a 200-ml plastic vial, and then introducing the host *S. borellii* always resulted in immediate parasitism. Wasp activity was stimulated by adding a heat source with a 500 w incandescent lamp for a few minutes at 30ºC before offering a host. Maintaining the wasps at a temperature of 27ºC was observed to increase their feeding activity; otherwise most of the wasps remained hidden in the plant or in the corners of the cage.

A few minutes after they were parasitized, the hosts were placed in moist sand inside the pipe-chambers and buried underground. The parasitized hosts were fed twice a

week with the same Purina cricket chow food used in the laboratory culture. To prevent hosts dying of dehydration, tap water was added if the sand in the chambers seemed dry.

Four wasp groups captured from the field were used to parasitize host mole crickets. Parasitism of all hosts was achieved in as few days as possible to reduce variability. Each group of parasitized hosts was considered a cohort (Table 7).

Table 7. Description of the cohorts of the wasp-parasitized hosts by numbers and dates.

Cohorts	Parasitized hosts[1]	Parasitism dates	
No.	No.	Initial	Last
1	65	22-VIII-2000	31-VIII-2000
2	38	1-IX-2000	11-IX-2000
3	28	30-IX-2000	1-X-2000
4	20	27-X-2000	

[1]*Scapteriscus borellii* adults.

Hosts of cohort 1 were randomly allotted to the first three sub-chambers (A, B and C). Hosts of cohorts 2 to 4 were randomly allotted to the four sub-chambers (A, B, C, and D).

The response variable was the time to develop for egg, larval, prepupal and pupal stages. The observation frequency varied as follows: egg development was recorded every six hours starting 24 hours after oviposition. Larval development was recorded daily starting five days after egg eclosion, continuing until the wasp grub reached a large size, or until the parasitic grub killed the host, then recordings were made every six hours until the beginning of the prepupal stage. Prepupal development was recorded every six hours until pupation was completed. Once pupation was completed, the metallic mesh dividing the sub-chambers was removed to allow the emerging wasps to dig up to the

surface. Pupal development was recorded daily, starting 20 days after pupation occurred, until adult emergence. Eventually, tardy pupae were visually inspected to assess whether they were still intact and to ensure the wasp had not escaped. Adult emergence was recorded by the presence of the adult wasp trying to escape from the chambers, but impeded by the fiberglass window-screen at the top of the pipe-chamber; daily inspection of the chambers was made during the experiments.

Results

The developmental times for stages and cohorts are presented, sorted by sub-chambers, stages and total development in Table 8, where the egg stage showed no difference among the sub-chamber depths. The larval stage showed no clear differences in development in cohorts 1 and 2, and no differences in cohort 3. The prepupal stage showed no difference in development related to sub-chamber depth in all the three cohorts evaluated. The pupal stage showed no difference among the sub-chambers where comparisons were possible. The total development showed statistical differences in cohorts 1 and 2. The differences observed in egg, larval and pupal stages were not highly significant. The mean values recorded in pupal and total development were numerically more contrasting among sub-chambers. In order to explain the possible differences in development among cohorts, the developmental times were pooled by cohort (without considering the sub-chamber) and presented in Table 9. The egg development was different among the three cohorts. Larval development in cohort 1 was highly different from that in cohorts 2 and 3. The prepupal stage showed no difference among cohorts. Pupal and total development showed no statistical difference among cohorts even though the mean values were very distinct, particularly in cohort 1, which was three times less than in cohort 3. The lack of differences in the pupal and total development is highly

related to the high variability of the respective estimates, which may mask the possible differences.

Table 8. Development time of *Larra bicolor* at various depths underground in the field at various dates in Gainesville, FL (29°40'N).

Cohort 1	Egg[a,b]		Larva[a,b]		Prepupa[a,b]		Pupa[a,b]		Total[a,b]	
Depths	n	Mean ± SEM	n	Mean ± SEM	n	Mean ± SEM	n	Mean ± SEM	n	Mean ± SEM
A	11	3.81 ± 0.12 a	10	9.18 ± 0.28 a	11	0.78 ± 0.09 a	2	44.23 ± 3.3 a	2	58.42 ± 3.4 a
B	8	3.65 ± 0.11 a	7	8.85 ± 0.27 ab	9	0.79 ± 0.09 a	2	45.31 ± 3.6 a	2	57.89 ± 3.9 a
C	10	3.60 ± 0.14 a	9	8.17 ± 0.19 b	13	0.87 ± 0.09 a	4	104.95 ± 62.9 a	4	117.32 ± 62.8 b

Cohort 2	Egg[a,b]		Larva[a,b]		Prepupa[a,b]		Pupa[a,b]		Total[a,b]	
Depths	n	Mean ± SEM	n	Mean ± SEM	n	Mean ± SEM	n	Mean ± SEM	n	Mean ± SEM
A	10	3.33 ± 0.1 a	2	11.02 ± 0.69 a	1	0.58 ± 0.0	0		0	
B	12	3.36 ± 0.1 a	3	11.18 ± 0.26 a	3	0.60 ± 0.1 a	1	238.00 ± 0.0 a	3	246.81 ± 3.83
C	4	3.33 ± 0.1 a	1	9.96 ± 0.00	1	0.50 ± 0.0	0		1	247.71 ± 0.00
D	3	3.56 ± 0.2 a	2	11.40 ± 1.94a	2	0.67 ± 0.2 a	2	160.89 ± 69.9 a	1	112.92 ± 0.00

Cohort 3	Egg[a,b]		Larva[a,b]		Prepupa[a,b]		Pupa[a,b]		Total[a,b]	
Depths	n	Mean ± SEM	n	Mean ± SEM	n	Mean ± SEM	n	Mean ± SEM	n	Mean ± SEM
A	6	3.89 ± 0.04 a	6	14.50 ± 0.43 a	5	0.87 ± 0.07 a	0		0	
B	2	3.96 ± 0.37 a	3	12.90 ± 0.49 a	2	0.79 ± 0.21 ab	0		0	
C	4	4.02 ± 0.11 a	4	13.38 ± 0.38 a	4	0.55 ± 0.03 b	2	210.27 ± 10.27	2	227.33 ± 10.46
D	2	3.94 ± 0.39 a	2	13.06 ± 1.02 a	2	0.54 ± 0.08 b	0		0	

Cohort 4	Egg[a,b]		Larva[a,b]		Prepupa[a,b]		Pupa[a,b]		Total[a,b]	
Depths	n	Mean ± SEM	n	Mean ± SEM	n	Mean ± SEM	n	Mean ± SEM	n	Mean ± SEM
A									0	
B									0	
C									2	198.75 ± 6.00
D									0	

[a]Mean days to complete development ± Standard error.
[b]Different letters among depths indicate statistical difference; Duncan's test when three or more means; 't' test when two means.

Table 9. Developmental time of four cohorts of *Larra bicolor* stages and total, averaged over all depths underground in the field in Gainesville, FL (29°40'N).

	Egg[a,b]		Larva[a,b]		Prepupa[a,b]		Pupa[a,b]		Total[a,b]	
Cohorts	n	Mean ± SEM	n	Mean ± SEM	n	Mean ± SEM	n	Mean ± SEM	n	Mean ± SEM
1	28	3.69 ± 0.07 b	26	8.74 ± 0.17 b	33	0.83 ± 0.05 a	8	74.86 ± 31.28 a	8	87.74± 31.17 a
2	29	3.35 ± 0.06 c	8	11.04 ± 0.43 a	7	0.60 ± 0.06 a	3	186.60 ± 45.44 a	5	220.21± 26.91 a
3	14	3.95 ± 0.07 a	15	13.10 ± 0.74 a	13	0.71 ± 0.06 a	2	210.27 ± 10.27 a	2	227.33± 10.46 a
4		ne		ne		ne		ne	2	198.75 ± 6.00 a

[a]Mean days to complete development ± Standard error.
[b]Different letters among cohorts indicate statistical difference; Duncan's test when three or more means.
ne = not evaluated.

The mean total developmental values per cohort (from Tables 8 and 9) are grouped in Table 10, where three developmental time groups are proposed, naming them as short, intermediate and long developmental times. Cohort 1, the summer wasps, produced all of the short total development, 56.59 d (all emerged by 18-24 Oct.), and one wasp that shifted to the long development, 305.8 d (emerged by 27 May). In Cohort 2, one wasp completed development in an intermediate time, 112.91 d (emerged by 30 Dec.), and 4 completed development in the long time, 247 d (all emerged by 8-27 May). Wasps from cohort 3 produced 2 individuals that developed in the long time, 227.3 d (emerged by 7 and 27 May). Cohort 4 produced 2 wasps that developed in the long time, 198.5d (emerged 9 and 21 May). The duration of the total development is presented with 99% confidence limits, with the idea to stress that the proposed groups do not overlap each other. Those limits demonstrate the pertinence of grouping them in those three categories that proves they are statistically different (Table 10).

Table 10. Trimodal total developmental time of *Larra bicolor* per cohort underground in the field in Gainesville, FL (29°40'N).

	Short[a,b]		Intermediate[a]		Long[a]	
Cohorts	n	Mean ± SEM	n	Mean ± SEM	n	Mean ± SEM
1	7	56.59 ± 1.43			1	305.79 ± 0.00
2			1	112.91 ± 0.00	4	247.03 ± 2.72
3					2	227.33 ±10.46
4					2	198.75 ± 6.00

[a]Mean days to complete development ± SEM.
[b]99% CL = 4.32 d (52.27-60.91).

Table 11 presents the individuals grouped by their corresponding life cycle stages and total developmental times, noting statistical differences between larval, pupal and total development, among the three life cycle developments.

Table 11. Trimodal life cycle developmental time of *Larra bicolor* underground in field conditions of Gainesville, FL (29°40'N).

	Short[a,b,c]		Intermediate[a,b,c]		Long[a,b,c]	
Stage	n	Mean ± SEM	n	Mean ± SEM	n	Mean ± SEM
Egg	7	3.49 ± 0.39 a	1	3.12 ± 0.00 a	7	3.49± 0.32 a
Larva	7	8.86 ± 0.84 a	1	13.33 ± 0.00 b	5	11.66 ± 3.19 ab
Prepupa	7	0.83 ± 0.31 a	1	0.45 ± 0.00 a	5	0.64± 0.25 a
Pupa	7	43.59 ± 3.32 a	1	96.00 ± 0.00 b	5	235.61± 43.78
Total	7	56.59 ± 3.49 a	1	112.91 ± 0.00 b	9	238.45± 25.21

[a]Mean days to complete development.
[b]Different letters between cycles indicate statistical difference. Duncan's test when three or more means
[c]Only wasps that completed the life cycle were included.

Survivorship

The number of wasp parasitic individuals that survived through the life cycle stages and their corresponding adult sexes per cohort are presented in Table 12. An overall 58% of the wasps reached the prepupal stage, which is an indicator that the developing larva killed the host mole cricket. An overall 47 to 49% of the wasps reached the pupal stage. An overall 13 to 14% of wasps completed development to the adult stage, with an overall sex ratio not statistically different from 50% (X^2 test).

Table 12. Number of individuals, corresponding proportion and sex ratio per cohort and overall of *Larra bicolor* developing on *Scapteriscus borellii* underground at field varying-temperatures.

Cohort	Eggs	Larvae[1]	Prepupae[1]	Pupae[1]	Adults[1]	Sexes
1	65	45 (69.2)	40 (61.5)	33 (50.8)	10 (15.4)	5M 3F
2	38	34 (89.5)	17 (44.7)	16 (42.1)	5 (13.2)	3M 2F
3	28	19 (67.9)	19 (67.9)	15 (53.6)	2 (7.1)	2F
4	20	ne	ne	7 (35.0)	2 (10.0)	2F
Overall[2]	131	98 (74.8)	76 (58.0)	64 (48.8)	17 (13.0)	8M 7F
Overall[3]	151			71 (47.0)	19 (14.5)	8M 9F

[1]Total number of individuals (percentage inside of parentheses).
[2]Considering cohorts 1 – 3 only.
[3]Considering all 4 cohorts.
ne = not evaluated.

The proportion of individuals entering the next stage is presented in Figure 6, where the declining populations followed the type III curve explained by Southwood (1978), where mortality occurs gradually by stage, being similar among the three cohorts.

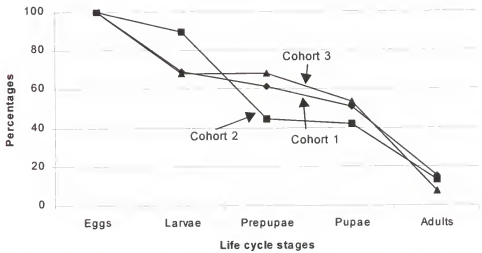

Figure 6. Proportion of individuals from three cohorts entering the life cycle stages of *Larra bicolor* developing on *Scapteriscus borellii* underground at field-varying temperatures.

The overall observed sex ratio in this work (which coincides with the observed ratio from constant temperature experiments) is somewhat intriguing, because during the three wasp seasons I scouted for wasps at patches of the nectar-plant *S. verticillata* (which included various times of the day and various periods of the year) the sex ratio of feeding wasps was always male-biased, most commonly seen as 3 to 10 males per one female, but sometimes reaching the exorbitant ratio of 100:1 M:F, or higher.

Discussion

The development of egg, larval, prepupal and pupal stages and thus the total development of the wasp *Larra bicolor* were evaluated under field conditions underground at three or four depths.

The mean developmental times of each cohort at the various depths showed few differences, and in most of the cases there was no difference (Table 8). A comparison of the development of the cohorts showed statistical differences in egg and larval development among cohorts. No differences in prepupal and pupal development were seen (Table 9). Development from egg to prepupa did not vary according to depth in the soil (Table 8).

The pupal stage was the most sensitive to seasonal changes (shown by the four cohorts that developed at various dates), showing three distinct durations of development time (Tables 10 and 12). The pupal (thus the total) development was classified as short, intermediate and long.

The capability of the pupal stage to diapause confers on this parasitoid the opportunity to survive the winter when low temperatures may occur and when the food plant, *Spermacoce verticillata*, may freeze to the ground. Most of the parasitoids that showed long life cycle emerged early and middle May, coinciding with two main factors that favor the parasitoid's survival: a) the food plant starts blooming in early May. This information is based on personal observations made during 1998 to 2001 b) the mole cricket populations are composed of old adults and small and small-medium size mole cricket nymphs. Adult and medium-large nymph host sizes are attacked by this wasp parasitoid during late May, as shown in Appendix B.

The life cycle duration of *Larra bicolor* was observed to include three distinct development times, where the pupal stage accounted for the variation observed in life cycles as follow: a short duration of 57 d (646 DD), an intermediate duration of 113 d (838 DD), and a long duration of 238 d (1093 DD), (see DD calculation on page 32, and

sample size on Table 10). The short cycle occurred in August. A possible seasonal strategy is proposed here to account for ability of the wasp to survive under the environmental condition of Gainesville, FL (29º40'):

Overwintered wasps. These wasps spend the winter as pupae from which adults begin to emerge in May. In May, these newly-emerged adults will find the preferred nectar- source plant (*Spermacoce verticillata*) blooming (in the localities where it occurs), providing energy to the wasps for mating, and for the females to hunt for host mole crickets. In May, these females will encounter overwintered *Scapteriscus vicinus* and *S. borellii* mole crickets that will be in the adult stage, providing suitable hosts, although by May they will not be especially abundant because they are undergoing attrition by aging.

First new generation. If the progeny of the overwintered generation of wasps (1. above) complete their life cycle:

i. In a short time (57 d), the adults will begin to emerge in July, a time when very few hosts of suitable size are available. This would be a poor strategy.

ii. In an intermediate time (113 d), they will begin to emerge in September, a time when the new generation of mole crickets exists as medium-sized to large-sized nymphs and is abundant (contrasted with numbers of survivors in May). Undersized hosts are not ideal because they may provide inadequate nutrition for wasp larvae. The longer a female wasp can survive, the greater is her opportunity for encountering large hosts. This appears to be the best strategy.

iii. In a long time (238 d), they will begin to emerge in December, which risks encountering freezing temperatures.

The optimal strategy for the progeny of the overwintered wasps (first new generation) is a development time of intermediate length (113 d).

Second new generation. For those wasps (first new generation) whose adults emerged in September, the strategies are to develop:

i. In a short time (57d), to provide a second new generation with adults emerging in November. This strategy would be highly successful except in years when an early freeze occurs.

ii. In an intermediate time (113 d), to give rise to adults emerging in January-February. This would be a disastrous strategy, because emerging adults would have little or no access to nectar.

iii. In a long time (238 d), to give rise to adults emerging in May of the following year. This would be successful.

That three distinct developmental times of *L. bicolor* should have been detected is remarkable. All three times (short, intermediate, and long) are needed to optimize its adaptation to the climate of northern Florida. The ability to adapt to the climate of northern Florida must have arisen during the evolution of this species in South America, where the species is widespread. The ability of the wasp to switch between these strategies is a tribute to its phenotypic plasticity. A simpler strategy would have been one with a single annual generation matched to the maximal annual abundance of hosts of suitable size: in other words, an annual cycle with presence of adult wasps only in September-November would have been easier.

Two types of questions are now presented. The basic question is: How is the plasticity of developmental time triggered and maintained? The applied question is: How

far north in the USA can *L. bicolor* establish a population in the presence of *Scapteriscus* mole crickets, and will it adopt a life cycle of a single annual generation at its northern limits (as contrasted with the three generations it supports in northern Florida)? It seems obvious that a single annual generation of this wasp would have less effect on mole cricket populations than do the three generations that seem evident in northern Florida.

In the tropics, in areas where *Scapteriscus* mole crickets breed continuously, *L. bicolor* might likewise breed continuously, so that the short (57 d) life cycle might maximize its reproductive strategy. In more temperate areas, other kinds of life cycles, adapted to breeding periods of *Scapteriscus* mole crickets and to availability of nectar, are surely more appropriate. At the extreme northern (and southern!) limit of the range of this wasp, wherever that may be, a single annual generation may be most appropriate to maximize its population size. In the intermediate (subtropical and almost-temperate) areas, other strategies may be better to maximize population size. This wasp, *L. bicolor*, is exceptional in having the ability to adapt its development time to maximize its population size.

This is the first work describing the life cycle of *Larra bicolor* under field conditions. The results present an insight into the performance of this parasitoid in adapting its populations to survive the climate of north Florida (29°40'N). The data presented support the hypothesized cold hardiness of this strain and reaffirm the potential of this parasitoid to establish and disperse its populations at this latitude and perhaps beyond toward other states where *Scapteriscus* mole crickets are already established.

Degree-day model predicting the development of the wasp *Larra bicolor*

Introduction

The rate of insect development depends upon the temperature to which the insects are exposed. For a developing insect, the rate at which time passes depends on the combination of its temperature-dependent development rate curve and the temperature at which it lives. The development rate curve may be altered by many factors of the environment, such as humidity, nutrition, or disease, but the curve always remains the same (Taylor 1981, Vinson and Iwantsch 1980). For each species, the temperature below that at which no measurable development occurs is the threshold of development.

The degree-day is a model that has been in use for more than 250 years (Wagner et al. 1984b) describing temperature/development relationship in the species (Honek 1999). The degree-day model is a linear approximation that enables calculating two virtual constants, lower threshold and sum of effective temperatures (an amount of heat needed for completing a development stage). This model fit the development rates within the range of ecologically relevant temperatures (Honek 1999, Campbell et al. 1974). The degree-day model does not work for all species. Hagstrum and Milliken (1991), Tanigoshi et al. (1976) and Logan et al. (1976) indicate this model is particularly invalid for mite populations. Wagner et al. (1984, and 1985) reviewed the efficiency of using constant temperatures to model development at variable temperatures. Hagstrum and Hagstrum (1970), Manel and Debouzie (1997b), showed how variable the results can be. Liu and Meng (1999) indicates that the degree-day model can simulate the development of *Myzus persicae* (Sulzer) very well when temperature does not go below 4°C. The degree-day model also works for *Cydia pomonella* (L.) (Howell and Neven 2000).

Wagner et al. (1984b) report that insects reared under identical conditions develop at different rates due to inherent differences among individuals in a population. Hagstrum and Hagstrum (1970) demonstrate that numerous insects develop at much lower temperatures when fluctuating diurnally than at a comparable mean constant temperature.

Understanding the relationship between temperature and development is important in constructing computer models for simulating biological control of pest populations (Lysyk 2000).

Scapteriscus mole crickets are pests of pasture- and turf-grasses and vegetables in the southeast and are dispersing westward in the USA. They are soil-inhabiting insects, reaching the ground surface for dispersal and mating purposes. Their life cycles are described in Chapter 2. The parasitoid wasp *Larra bicolor* is a natural enemy of *Scapteriscus* mole crickets, and has been released in the USA.

The objective of this work was to evaluate the degree-day model based upon constant temperatures to predict the field development of *Larra bicolor* parasitizing mole crickets in north Florida (29°40'N).

Materials and Methods

A set of experiments quantifying developmental time of *Larra bicolor* per stage and total was performed at seven constant temperatures in incubation chambers. The developmental time of *L. bicolor* at field-varying temperatures was determined by studying the development of three wasp cohorts underground during summer, autumn and winter seasons. The parasitic wasp developed on *Scapteriscus borellii* and temperatures were recorded every 30 min at four soil depths.

Developmental rates of *L. bicolor* are calculated using the linear equation (Eq. 1) plotted against temperature. The temperatures at the most linear portion of the curve were selected to calculate their linear equations by simple linear regression analysis (Eq. 1).

$Y = a + b(x)$: (Eq.1)

Y = Developmental rate (1/days)
a = Intercept
b = Slope
x = Temperature ºC

The lower temperature threshold and thermal constant (degree-days) were calculated from the linear equation, using its regression coefficients where: lower threshold (t_0) = -(a/b) and thermal constant (k) = 1/b.

The degree-days required per individual and total to complete development at constant and field temperatures are calculated by the following equation:

$$Y = \sum_{i=1}^{n} T\,(t - t_0) \qquad \text{(Eq. 2)}$$

Y = Degree-days
T = Time (time step = 30 minutes = 1/48 days)
t = Temperature recorded every 30 min
t_0 = Lower temperature threshold (per corresponding stage or total)
n = Number of days for stage to develop

The validation of development of *L. bicolor* at constant temperatures as a predictor of development at field temperatures is evaluated by comparing degree-day data sets in the same graph to observe how well the degree-days at constant temperatures match degree-days at field varying temperatures. The statistical comparison of what can be seen from the constant and field temperature linear equations was done by comparison of slopes. The linear equations were calculated by regressing the observed DD with corresponding mean temperatures. Degree-days for development of individuals from the

constant temperature experiment used the temperature at which the incubation chamber was set. Degree-days for development of individuals from the field experiments were regressed against the mean temperature recorded during the period of time the development was achieved. All the individuals completing the respective stage were included. The null hypothesis was slopes equal to zero.

Results

The wasp *Larra bicolor* developing in incubation chambers completed its life cycle development at temperatures ranging from 22.5 to 32.5°C (only one wasp completed development at 22.5°C and was not included in the analysis), although a few eggs developed at 20°C and some eggs and larvae developed at 22.5°C.

Developmental times were transformed to rates then plotted against their respective temperature (Figure 2) showing the most linear development from 22.5 to 32.5°C for the egg stage, and from 22.5 to 30.0°C for all the other stages and total. The linear equation that best estimates development at constant temperatures, lower thresholds and degree-days is shown in Table 13.

The accumulated degree-days required to develop by stage at constant temperatures and at field-varying temperatures are shown in Table 14.

Degree-days required for pupal development of *Larra bicolor* underground at field-varying temperatures are presented in Figure 7. The differences in development are clear among the three groups. The pupae showing the short development, considered as the non-diapausing population, were the only ones included in this validation; the other two groups developed at temperatures outside the range studied.

Table 13. Parameter values for the linear model, lower thresholds and thermal constants (degree-days) for development of *Larra bicolor* per stage and total at constant temperatures.

Stage	Linear equation[a] Y = a + b (x)(Eq. 1)	Lower threshold °C t_0 = -(a / b)[b]	Thermal constant k^c = 1 / b
Egg	Y = -0.401469 0.022128(x)	18.143	45.44±1.45
Larva	Y = -0.221118 + 0.011875(x)	18.620	84.42±1.92
Prepupa	Y = -3.787811 + 0.203193(x)	18.641	5.05±0.34
Pupa	Y = -0.038899 + 0.002429(x)	16.010	413.13±11.59
Total	Y = -0.031264 + 0.001852(x)	16.770	541.67±12.41

Y = Developmental rate (Y = 1 / days), a = intercept, b = slope, x = temperature
t_0 = lower temperature threshold
k = Degree-days±SEM (days)

Table 14 Degree-days (days) required for *Larra bicolor* stages and total to develop at constant and field temperatures.

Temp.°C[a]	N	Egg[c]	N	Larva[c]	N	Prepupa[c]	N	Pupa[c]	N	Total[c]
25.0	26	43.61 ± 1.62	16	83.17 ± 3.27	16	4.9 ± 0.58	6	400.74 ± 21.17	6	525.8 ± 27.36
27.5	23	49.3.7 ± 2.46	12	87.19 ± 8.05	12	5.2 ± 1.59	6	442.13 ± 23.59	6	575.3 ± 26.27
30.0	27	44.34 ± 1.82	17	83.39 ± 3.03	17	4.9 ± 1.12	8	401.19 ± 22.78	8	527.9 ± 19.72
32.5	25	44.87 ± 1.38								
FIELD[b]	41	40.4 ± 1.17	47	89.7 ± 2.53	53	6.6 ± 0.85	8	481.6 ± 14.39	8	595.1 ± 16.38

[a]Temperatures in incubation chambers.
[b]Overall Mean ± SEM of all wasps developing during the summer in underground chambers in the field.
Mean ± SEM.

Data for all the individuals sorted by stage and total, whose complete development was recorded from field and from incubation chambers, are depicted in Figure 8. At most of the degree-days calculated, all the stages fell inside the observed range at constant temperatures. Slopes for constant and field temperature tend to be fairly flat, suggesting the degree-days required by stage are constant along the temperature range where development occurred.

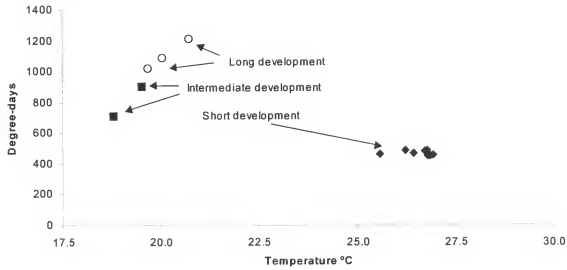

Figure 7. Degree-days required for pupal development of *Larra bicolor* underground at field-varying temperatures. Pupal stage grouped by kind of developmental times (short, intermediate, and long).

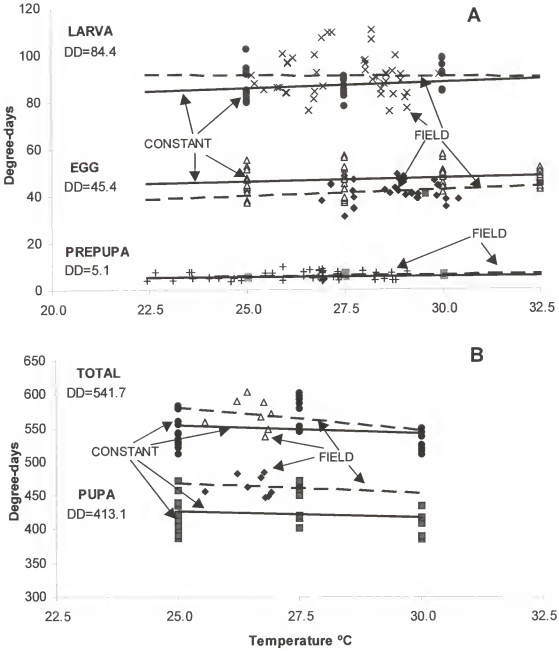

Figure 8. Comparison of degree-days required for *Larra bicolor* life stages and total to develop at constant and field varying temperatures. A) Egg, larval and prepupal development. B) Pupal and total development (short development only).

The slopes per stage and total were evaluated showing that none of the regression slopes were significantly different between field and constant temperature experiments (Table 15). These results support the hypothesis that data from constant temperatures can

estimate development in field-varying temperatures for *Larra bicolor* over the range of temperatures in these studies.

Table 15. Statistical significance of slopes in regression analysis of development of *Larra bicolor* stages and total, from the field and from incubation chambers.

Development	Egg	Larva	Prepupa	Pupa	Total
Field[a]					
p[c]	0.625 ns	0.283 ns	0.819 ns	0.829 ns	0.741 ns
Chambers[b]					
p[c]	0.126 ns	0.286 ns	0.295 ns	0.648 ns	0.574 ns

[a]. Development in field-varying temperatures

[b]. Development in incubation chambers at constant temperatures

[c] statistic of t-test

ns = statistically not significant

Discussion

Development under a regime of fluctuating temperatures often differs from that under constant temperatures. Higley et al. (1986) mention that the magnitude of the differences between developmental times under constant and fluctuating temperatures depends on the average temperature, amplitude and frequency of the fluctuations.

Larra bicolor development estimated at constant temperatures and evaluated as degree-days was a good estimator of development at field-varying temperatures. The coincidence of the constant temperature degree-day trend with observed development in the field (Figure 29) is the first clue to the efficiency of constant temperature development to predict field development. The lack of significant differences of slopes in regression analysis of all stages under the two conditions studied proves that *Larra bicolor* possesses a thermal constant that applies at the temperature range used in this study (Table 25). This thermal constant applies to the pupal stage when this develops in a short time, which mostly occurs during summer and early autumn.

One of the uses of the results presented in this paper is the possibility to predict the period of time when this parasitoid would be present, thus parasitizing host mole crickets in north Florida. The importance of having this knowledge will allow biologists and extension service personnel to predict when the parasitic wasp would emerge from the soil in spring. By this means the phenological model proposed in this paper can be integrated with the pest management of mole crickets. A direct benefit of this phenological model would be the timing of pesticide applications according to the predicted wasp season.

The use of the model is restricted to the soil temperature range from 25 to 32.5°C, which occurs from May to November or early December, which covers the normal wasp season. The soil temperature source can be the Florida Automated Weather Network, whose records proved highly coincident with soil temperature in the experimental area (at 22.5 cm depth, see appendix C) where development of the wasp was studied.

The development of *Larra bicolor* has been modeled based on constant temperature studies using the degree-day model approach. The model is a good predictor of development in north Florida soils.

The degree-day model to validate development of *Larra bicolor* at constant temperatures proved valuable to predict field-varying temperature development of non-diapausing populations. Extensive work would be required to model diapausing populations.

CHAPTER 4
DEVELOPMENT OF THE FLY *ORMIA DEPLETA*.

Development of *Ormia depleta* at constant temperatures

Introduction

The tachinid fly *Ormia depleta* was brought to Florida in 1987 as part of the mole cricket research program and considered potentially the most effective control agent against mole crickets (Fowler 1987, 1989, Frank et al. 1996). This endoparasitic fly, of crepuscular habits, locates host mole crickets by the male host's calling song and deposits larvae (planidia) on or near the host (Fowler 1987, Walker et al. 1996).

The first parasitoid strain ("old" strain, from 23°S in Brazil) released in Florida in 1988 (Frank et al. 1996), was considered as largely responsible for a decline in mole cricket populations (Parkman et al. 1996). By 1994, this "old" strain disappeared from the Gainesville area (29° 40'N latitude), perhaps due to its poor tolerance to cold and long winters (Walker et al. 1996). In recent years, in late autumn, a few flies have been captured near Gainesville, but these were considered to be individuals dispersing from south toward north without re-establishing populations.

In 1999 a second tachinid strain ("new" strain) was imported. It was hoped that this "new" strain, from 30°S in Brazil, might be more cold tolerant and could establish populations north of 29°N in Florida and other states (H. Frank personal communication).

Studies performed with the first strain of *Ormia depleta* focused on establishment and range (Frank et al. 1996), seasonality (Walker et al. 1996), effectiveness in

controlling mole crickets (Parkman et al. 1996), techniques for rearing it in the laboratory (Wineriter and Walker 1990) and food source (Welch 2000). This "old" strain was in culture from 1987 (Frank et al. 1996) until 1999, but it is the "new" strain that is now in culture to supply populations. Wineriter and Walker (1990) determined the life history of the first strain under laboratory conditions at 23-25ºC.

There is no information about the effect of temperature on life history or seasonal phenology. Limits of geographical expansion of *O. depleta* are poorly understood. The fact that the "old" introduced strain remained well established in south-central Florida (27ºN), but disappeared farther north suggests a strong influence of temperature regimes, where either colder winters kill the flies or cause the lack of adequate winter nectar sources (Walker et al. 1996). These arguments led to the introduction of a second strain with the hope that it would be more cold hardy and survive colder winters.

In recent years the efficiency of *O. depleta* has been questioned, Potter (1998) stated that this parasitoid does not seen to have a major effect on mole cricket populations, but controversy about the parasitoid role could be due to insufficient information on its biology (Ryoo et al. 1991).

Developmental times of many species of poikilotherms as a function of temperature have been described by regression equations (Wagner et al. 1984a). Such regression equations can provide a sample of calculating developmental times in insect population models or can be used for quantitative comparisons of the effect of temperature on the developmental times of different stages and species of insects (Hagstrum and Milliken 1988).

The work described here was aimed to determine the effect of temperatures on development and survivorship of the "new" strain of *Ormia depleta*, then compare the developmental times and developmental rates of the two strains.

Materials and Methods

The flies used in this research were laboratory-reared flies using the methods described by Wineriter and Walker (1990). The old strain flies were under culture until 1999 when the new strain was brought from South America.

Gravid fly females, recognized by a dark spot seen near the apex of the abdomen due to the presence of fully grown planidia, were dissected to expose planidia on wet paper in a petri dish. Only dark colored (mature first instar) planidia were used to parasitize host mole crickets. Three planidia were placed in the membranous tissue behind and underneath the posterior of a host pronotum, making sure those planidia remained there until the host was placed into its plastic cup (Wineriter and Walker 1990).

Parasitized hosts *S. borellii* were confined in a 200-ml plastic cup filled with wet sterilized sand. The cups were then transferred to culture chambers at eleven constant temperatures, 10.0, 15.0, 17.0 17.5. 20.0, 22.5, 25.0, 27.5, 30.0, 32.5, 35.0 ± 0.2°C. Relative humidity was set at 80%, and the photoperiod at 14: 10 (L: D). 15 newly parasitized hosts (three planidias per host) were placed per temperature-chamber.

Parasitized hosts were fed twice a week following the standard procedure for *S. abbreviatus* culture. Development was tracked by observing the host through the transparent cup walls every six hours, until parasitic exited the host (by making a hole from inside out of the host's abdomen, killing the host) to pupate in the sand. The pupal development was tracked at the same frequency until adult parasitoid emergence.

The response variables recorded per strain were larval and pupal development at each constant temperature. Survivorship and adult sex ratio were calculated at each temperature. Data were sorted by sex and pooled sexes, and comparisons were made between sexes. Developmental times were compared by "t-test" between temperatures. Developmental rates were compared using linear regression models. Degree-days and lower temperature thresholds were calculated from the linear equations. Finally these variables were tested between strains.

Results

The new strain

The host, *S. borellii*, did not survive more then seven days at temperatures of 10.0, 15.0 and 35.0ºC. The parasitoid was able to develop at temperatures ranging from 17.0 to 30.0ºC, where developmental time was inversely related to temperature, as is common in poikilothermic organisms. Developmental time for larvae ranged from 23.6 d to 6.9 d (pooled sexes), 21.7 to 6.8 (males) and 26.5 to 7.9 d (females). Development time for pupae ranged from 30.4 d to 9.3 d (pooled sexes), 31.0 d to 9.0 d (males) and 29.5 d to 9.35 d (females). Total development time ranged from 54 d to 16.5 d (pooled sexes), 52.7 d to 15.9 d (males), and 56 d to 16.5 d (females). (Tables 16,17 and 18 and Figure 9).

Mean development times were compared per sex by the 't-test' assuming equal variances (Table 19). The null hypothesis was set as males develop in less time **than** females at either stage or total development. No difference was observed at larval stage. Statistical differences were observed in the pupal stage where males developed in less time at 30.0, 25.0, 22.5, 20.5, and 17.5, thus reflecting the same differences in the total

development. Although statistically not different, males also developed faster than females at 27.5°C.

Table 16. Development of *Ormia depleta* (new strain) pooled sexes life stages.

Temp.		Days required to complete development[a]		
°C	n	Larva	Pupa	Total
30.0	37	6.91 ± 0.11 (41.9)	9.28 ± 0.08 (58.1)	16.48 ± 0.13
27.5	27	7.02 ± 0.09 (41.5)	9.90 ± 0.07 (58.5)	16.92 ± 0.08
25.0	47	8.61 ± 0.13 (43.4)	11.22 ± 0.20 (56.6)	19.82 ± 0.18
22.5	24	10.22 ± 0.19 (41.5)	14.43 ± 0.11 (58.5)	24.65 ± 0.27
22.0	14	12.43 ± 0.47 (44.7)	15.36 ± 0.13 (55.3)	27.79 ± 0.58
20.0	53	14.25 ± 0.19 (41.5)	20.11 ± 0.16 (58.5)	34.35 ± 0.20
17.5	17	20.98 ± 0.38 (41.6)	29.45 ± 0.18 (58.4)	50.43 ± 0.49
17.0	5	23.60 ± 1.25 (43.7)	30.40 ± 0.40 (56.3)	54.00 ± 0.89

[a] Mean ± SEM; porcentage of developmental time required for each life stage given in parenthesis.

Table 17. Development of *Ormia depleta* (new strain) male stages.

Temp.		Days required to complete development[a]		
°C	n	Larva	Pupa	Total
30.0	18	6.82 ± 0.14 (43.0)	9.06 ± 0.11 (57.0)	15.88 ± 0.16
27.5	14	7.10 ± 0.13 (42.2)	9.86 ± 0.10 (57.8)	16.96 ± 0.13
25.0	23	8.64 ± 0.20 (41.7)	10.87 ± 0.27 (58.3)	19.51 ± 0.30
22.5	14	9.90 ± 0.19 (40.6)	14.14 ± 0.10 (59.4)	24.04 ± 0.27
20.0	27	14.15 ± 0.23 (40.2)	19.64 ± 0.21 (59.8)	33.77 ± 0.26
17.5	5	20.78 ± 0.53 (39.4)	28.68 ± 0.28 (60.6)	49.46 ± 0.72
17.0	3	21.67 ± 0.67 (38.8)	31.00 ± 0.00 (61.2)	52.67 ± 0.67

[a] Mean ± SEM; percentage of developmental time required for each life stage given in parenthesis.

Table 18. Development of *Ormia depleta* (new strain) female stages.

Temp.		Days required to complete development[a]		
°C	n	Larva	Pupa	Total
30.0	19	6.99 ± 0.16 (43.3)	9.49 ± 0.11 (56.7)	16.47 ± 0.19
27.5	13	6.93 ± 0.12 (41.3)	9.95 ± 0.10 (58.7)	16.88 ± 0.11
25.0	24	8.58 ± 0.19 (40.7)	11.56 ± 0.27 (59.3)	20.13 ± 0.20
22.5	10	10.66 ± 0.33 (40.9)	14.85 ± 0.14 (59.1)	25.51 ± 0.41
20.0	23	14.36 ± 0.36 (40.4)	20.47 ± 0.22 (59.6)	34.83 ± 0.30
17.5	12	21.06 ± 0.51 (38.8)	29.77 ± 0.14 (61.2)	50.83 ± 0.61
17.0	2	26.5 ± 0.5 (38.8)	29.50 ± 0.5 (61.2)	56.00 ± 0.00

[a] Mean ± SEM; percentage of developmental time required for each life stage given in parenthesis.

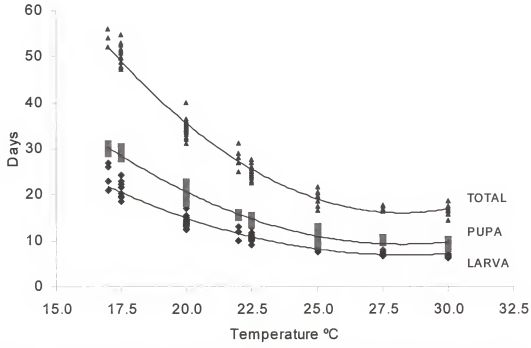

Figure 9. Development time of *Ormia depleta* "new" strain, pooled sexes, at constant temperatures.

Survivorship recorded from each temperature is presented in Figure 10, where maximum survival occurred at 27.5°C, followed by 22.5, 20.0, and 25.0°C in a group with survivorship greater than 60% and greater than 50 % at 17.5°C. The greater mortality occurred in the larval stage.

Table 19. T-test comparison of developmental time (days) of *Ormia depleta* (new strain) between sexes per temperature per stage and total life cycle.

	Larva		Pupa		Total	
Temp. °C	Male	Female	Male	Female	Male	Female
30.0	6.82 a	6.99 a	9.06 a	9.49 b	15.88 a	16.47 b
27.5	7.10 a	6.63 a	9.86 a	9.95 a	16.96 a	16.88 a
25.0	8.64 a	8.58 a	10.87 a	11.56 b	19.51 a	20.13 b
22.5	9.90 a	10.66 a	14.14 a	14.85 b	24.04a	25.51 b
20.0	14.15 a	14.36 a	19.64 a	20.47 b	33.77 a	34.83 b
17.5	20.78 a	21.06 a	28.68 a	29.77 b	49.46 a	50.83 b
17.0	21.67 a	26.50 a	31.00 a	29.50 b	52.67 a	56.00 b

[a] Means followed by the same letter are not statistically different (one tailed test)

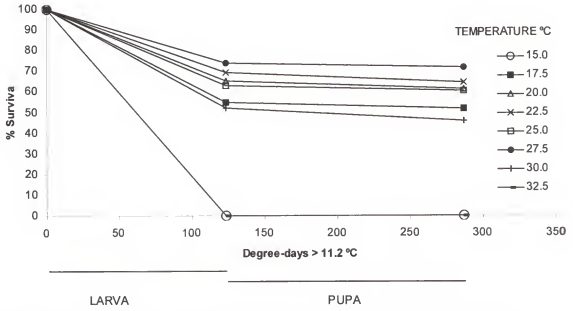

Figure 10. Survivorship of *Ormia depleta* "new" strain, pooled sexes, at constant temperatures.

Regression parameters corresponding to each data set are shown in Tables 20,21 and 22 and in Figure 11. The coefficients of determination ranged from 0.90 to 0.95 representing the good fit of all models. The lower threshold values ranged from 11.1 to 11.6. The accumulated degree-days required to complete development per stage and total are shown in last column of Tables 20, 21 and 22.

Table 20. Linear regression models (Y= developmental rate, x= temperature), lower developmental thresholds (t), and degree-days required for *Ormia depleta* (new strain) pooled sexes life stages to develop.

Stage	y Intercept ± SEM	Slope ± SEM	R^2 ± SE	*t* (°C)	Degree-days (Days ± SE)
Larva	-0.092 ± 0.0044	0.0082 ± 0.0002	0.90 ± 0.0109	11.2	123.4 ± 0.9
Pupa	-0.071 ± 0.003	0.0062 ± 0.0001	0.92 ± 0.0076	11.5	163.6 ± 0.9
Total	-0.04 ± 0.0013	0.0035 ± 0.00006	0.95 ± 0.0033	11.4	286.6 ± 1.3

Table 21. Linear regression models (Y= developmental rate, x= temperature), lower developmental thresholds (t), and degree-days required for *Ormia depleta* (new strain) male stages to develop.

Stage	y Intercept ± SEM	Slope ± SEM	R^2 ± SE	*t* (°C)	Degree-days (Days ± SE)
Larva	-0.090 ± 0.0061	0.0082 ± 0.0003	0.90 ± 0.0106	11.1	124.0 ± 1.2
Pupa	-0.074 ± 0.0044	0.0063 ± 0.0002	0.91 ± 0.0077	11.6	159.5 ± 1.2
Total	-0.041± 0.002	0.0036 ± 0.00008	0.95 ± 0.0034	11.4	283.2 ± 1.8

Table 22. Linear regression models (Y= developmental rate, x= temperature), lower developmental thresholds (t), and degree-days required for *Ormia depleta* (new strain) female stages to develop.

Stage	y Intercept ± SEM	Slope ± SEM	R^2 ± SE	*t* (°C)	Degree-days (Days ± SE)
Larva	-0.094 ± 0.0063	0.0083 ± 0.0003	0.90 ± 0.0113	11.4	122.9 ± 1.4
Pupa	-0.069 ± 0.004	0.0061 ± 0.0002	0.92 ± 0.0073	11.5	167.4 ± 1.3
Total	-0.040 ± 0.0018	0.0035 ± 0.00007	0.95 ± 0.0032	11.5	289.6 ± 1.8

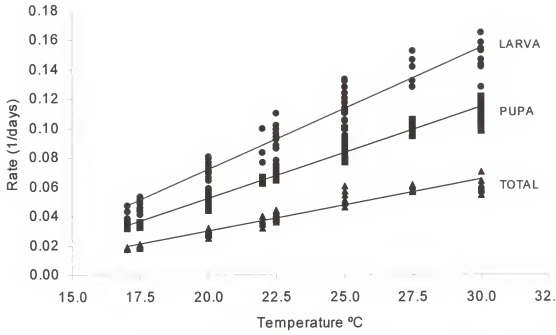

Figure 11. Developmental rates of *Ormia depleta* "new strain", pooled sexes, at constant temperatures.

The old strain

This old strain developed at temperatures ranging from 17.5 to 30.0°C, showing an inverse relationship between developmental time and temperature. Development time for larvae ranged from 20.39 d to 6.65 d (pooled sexes), 20.52 d to 6.40 d (males) and 20.13 d to 6.78 d (females). Development time for pupae ranged from 32.13 d to 8.72 d (pooled sexes), 31.58 d to 8.48 d (males) and 33.23 d to 8.84 d (females). Total development time ranged from 52.52 to 15.38, 52.10 to 14.88, and 53.35 to 15.63 d (females). (Tables 23, 24 and 25 and Figure 12).

Table 23. Development of *Ormia depleta* (old strain) pooled sexes life stages.

Temp.		Days required to complete development[a]		
°C	n	Larva	Pupa	Total
30.0	6	6.40 ± 0.27 (43.0)	8.48 ± 0.06 (57.0)	14.88 ± 0.21
27.5	13	7.15 ± 0.31 (42.2)	9.78 ± 0.37 (57.8)	16.93 ± 0.30
25.0	24	7.95 ± 0.16 (41.7)	11.13 ± 0.11 (58.3)	19.08 ± 0.17
22.5	9	9.00 ± 0.42 (40.6)	13.17 ± 0.30 (59.4)	22.17 ± 0.26
20.0	15	13.83 ± 0.48 (40.2)	20.56 ± 0.41 (59.8)	34.39 ± 0.29
17.5	6	20.52 ± 0.40 (39.4)	31.58 ± 0.96 (60.6)	52.10 ± 1.01

a Mean ± SEM; percentage of developmental time required for each life stage given in parenthesis.

Table 24. Development of *Ormia depleta* (old strain) male life stages.

Temp.		Days required to complete development[a]		
°C	n	Larva	Pupa	Total
30.0	6	6.78 ± 0.11 (43.3)	8.84 ± 0.14 (56.7)	15.63 ± 0.21
27.5	13	6.73 ± 0.32 (41.3)	9.89 ± 0.27 (58.7)	16.62 ± 0.52
25.0	24	7.77 ± 0.10 (40.7)	12.02 ± 0.18 (59.3)	19.78 ± 0.20
22.5	9	9.67 ± 0.00 (40.9)	13.58 ± 0.33 (59.1)	23.35 ± 0.33
20.0	15	14.23 ± 0.40 (40.4)	20.98 ± 0.18 (59.6)	35.21 ± 0.40
17.5	6	20.13 ± 0.0 (38.8)	33.23 ± 0.23 (61.2)	53.35 ± 0.23

a Mean ± SEM; percentage of developmental time required for each life stage given in parenthesis.

Table 25. Development of *Ormia depleta* (old strain) female life stages.

Temp.		Days required to complete development[a]		
°C	n	Larva	Pupa	Total
30.0	6	6.65 ± 0.13 (43.3)	8.72 ± 0.12 (56.7)	15.38 ± 0.21
27.5	13	6.93 ± 0.22 (41.3)	9.84 ± 0.21 (58.7)	16.76 ± 0.30
25.0	24	7.88 ± 0.10 (40.7)	11.50 ± 0.13 (59.3)	19.38 ± 0.15
22.5	9	9.22 ± 0.29 (40.9)	13.31 ± 0.23 (59.1)	22.53 ± 0.27
20.0	15	14.10 ± 0.30 (40.4)	20.84 ± 0.18 (59.6)	34.94 ± 0.30
17.5	6	20.39 ± 0.26 (38.8)	32.13 ± 0.70 (61.2)	52.52 ± 0.70

a Mean ± SEM; percentage of developmental time required for each life stage given in parenthesis.

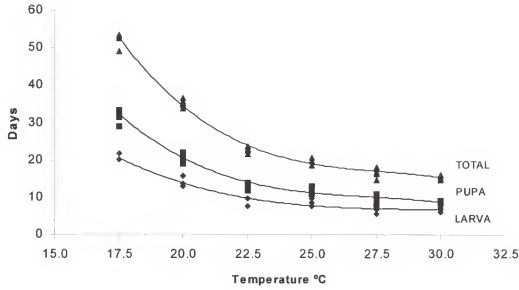

Figure 12. Developmental time of *Ormia depleta,* old strain, life cycle at constant temperature.

Mean developmental times were compared between sexes per temperature per stage by the 't-test' assuming equal variances (Table 26). The null hypothesis was that males develop in less time than females at either temperature per stage or total development. No difference was observed in the larval stage. In the pupal stage the only difference was observed at 25.0°C. In the total life cycle differences were observed at 30.0, 25.0, and 22.5°C. At all cases where differences were observed, males developed in less time than females.

Survival recorded from each temperature is presented in Figure 13, where maximum survival (87-88%) occurred at 22.5 and 25.0°C, followed by 27.5, 20.0, 30.0 and 17.5°C respectively, in a group with survivorship ranging from 50% to 82%. The greater mortality occurred in the larval stage at all temperatures but 22.5°C.

Table 26. T-test comparison of developmental time (days) of *Ormia depleta* (old strain) between sexes per temperature per stage and total life cycle.

	Larva[a]		Pupa[a]		Total[a]	
Temp. °C	Male	Female	Male	Female	Male	Female
30.0	6.82 a	6.99 a	9.06 a	9.49 b	15.88 a	16.47 b
27.5	7.10 a	6.63 a	9.86 a	9.95 a	16.96 a	16.88 a
25.0	8.64 a	8.58 a	10.87 a	11.56 b	19.51 a	20.13 b
22.5	9.90 a	10.66 a	14.14 a	14.85 b	24.04a	25.51 b
20.0	14.15 a	14.36 a	19.64 a	20.47 b	33.77 a	34.83 b
17.5	20.78 a	21.06 a	28.68 a	29.77 b	49.46 a	50.83 b
17.0	21.67 a	26.50 a	31.00 a	29.50 b	52.67 a	56.00 b

[a] Means followed by the same letter are not statistically different (one tailed test)

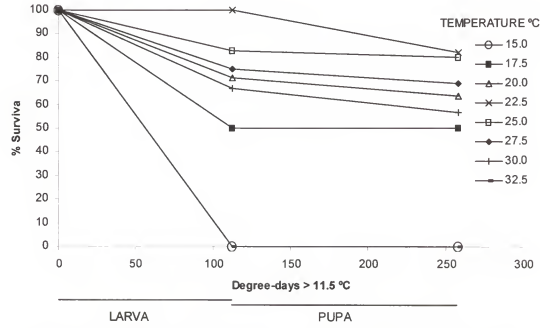

Figure 13. Survivorship of *Ormia depleta* old strain, pooled sexes, at constant temperatures.

Regression parameters corresponding to each data set are shown in Tables 27, 28 and 29 and in Figure 14. The coefficients of determination ranged from 0.88 to 0.97 representing a good fit of all models. The lower threshold values ranged from 11.3 to

12.7. The accumulated degree-days required to complete development per stage and total are shown in last column of Tables 27, 28 and 29.

Table 27. Linear regression models (Y= developmental rate, x= temperature), lower developmental thresholds (t), and degree-days required for *Ormia depleta* (old strain) life stages to develop.

Stage	y Intercept ± SEM	Slope ± SEM	R^2 ± SE	*t* (°C)	Degree-days (Days ± SE)
Larva	-0.104 ± 0.0097	0.009 ± 0.0004	0.88 ± 0.0121	11.5	112.4 ± 1.4
Pupa	-0.087 ± 0.0048	0.0069 ± 0.0002	0.95 ± 0.0059	12.6	146.1 ± 1.3
Total	-0.048 ± 0.0026	0.0039 ± 0.0001	0.95 ± 0.0033	12.2	258.2 ± 2.1

Table 28. Linear regression models (Y= developmental rate, x= temperature), lower developmental thresholds (t), and degree-days required for *Ormia depleta* (old strain) male life stages to develop.

Stage	y Intercept ± SEM	Slope ± SEM	R2 ± SE	*t* (°C)	Degree-days (Days ± SE)
Larva	-0.101 ± 0.0137	0.009 ± 0.0006	0.88 ± 0.0117	11.3	113.1 ± 2.0
Pupa	-0.09 ± 0.0071	0.0071± 0.0003	0.94 ± 0.006	12.6	142.1 ± 1.8
Total	-0.048 ± 0.0034	0.004 ± 0.0001	0.96 ± 0.0029	12.1	254.7 ± 3.0

Table 29. Linear regression models (Y= developmental rate, x= temperature), lower developmental thresholds (t), and degree-days required for *Ormia depleta* (old strain) female life stages to develop.

Stage	y Intercept ± SEM	Slope ± SEM	R^2 ± SE	*t* (°C)	Degree-days (Days ± SE)
Larva	-0.106 ± 0.014	0.0090 ± 0.0006	0.88 ± 0.0128	11.7	111.6 ± 1.9
Pupa	-0.086 ± 0.0054	0.0068 ± 0.0002	0.97 ± 0.0049	12.7	148.3 ± 1.4
Total	-0.048 ± 0.0034	0.0039 ± 0.0002	0.95 ± 0.0034	12.3	259.7 ± 2.8

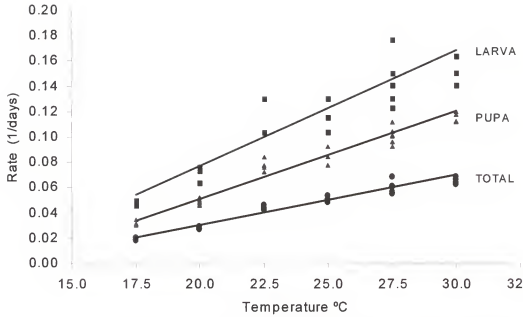

Figure 14. Developmental rates of *Ormia depleta* old strain, pooled sexes, life cycle at constant temperatures.

Discussion

Ormia depleta development rates were sensitive to temperatures and showed a linear response at temperatures ranging from 17.5 to 30.0°C (Figures 11 and 14). The developmental times reported by Wineriter and Walker (1990) coincided with those reported in this study, for both strains, at temperatures between 22.5 and 25.0°C for each sex and pooled. Larval development was shorter than pupal development at all temperatures, maintaining similar proportions among temperatures (Tables 16-18, 23-25). Wineriter and Walker (1990) observed that emergence pattern was significantly different for the two sexes, with male adults generally emerging first. The difference in development by sex was more evident in the new strain than the old strain as indicated in Tables 19 and 23, respectively.

Comparing the development of the two strains (Figures 15,16 and 17), male larval development times were statistically different only at 25.0ºC. For females and pooled sexes there were differences at 22.5 and 25.0ºC. Pupal development for males, females and pooled sexes showed differences at 17.5, 22.5 and 30.0ºC. Male total development times were different at 25.0ºC, and females and pooled sexes showed differences at 17.5, 22.5 and 30.0ºC. The difference in developmental time between sexes was more evident in the new strain than the old, where the larval stage was less variable than pupal and total between the two strains.

The development time per strain at the extreme temperatures was statistically different. The old strain took more time to develop at 17.5ºC and the new strain took more time at 30.0ºC (more evident at females, Figure 18, and pooled sexes, Figure 16). In other words, for pupal and total development, the new strain developed faster than the old strain at the lower temperature, but it was the old strain that developed faster than the new strain at the higher temperature.

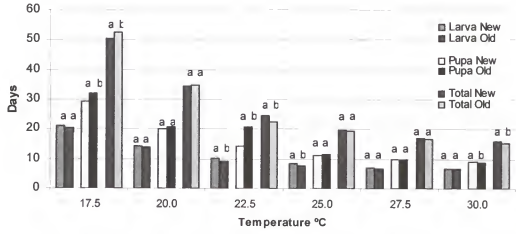

Figure 15. Developmental times of two strains (new and old) of *Ormia depleta*, pooled sexes, at constant temperatures. Means followed by different letter are statistically different ("t – test", $P<0.05$).

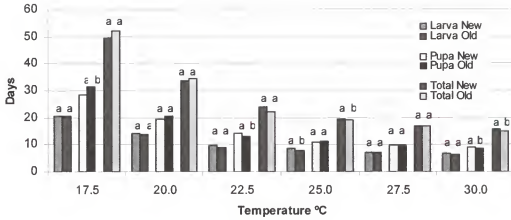

Figure 16. Developmental times of two strains (new and old) of *Ormia depleta* males at constant temperatures. Means followed by different letter are statistically different ("t – test", P<0.05).

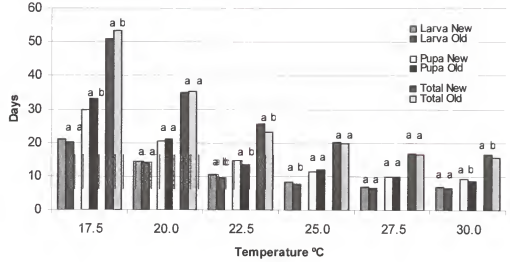

Figure 17. Developmental times of two strains (new and old) of *Ormia depleta* females at constant temperatures. Means followed by different letter are statistically different ("t – test", P<0.05).

Temperatures at which this parasitoid may develop were identified (Figures 9 and 12). The rate of increase decreased at 30ºC and stopped at 32.5ºC. the upper temperature

threshold must fall between these temperatures. The survivorship observed for the two strains was statistically different only at 25°C.

The developmental rates of both strains were compared by means of their corresponding linear regression models shown in Figures 11 and 14. This comparison was performed to determine whether both lines can be considered to be estimates of a common ß (slope) following the procedure of Steel and Torrie (1960: 173), Sokal and Rohlf (1995). The results established that the two strains develop differently at constant temperatures (Table 30). The new strain developed slower than old strain at higher temperatures, but faster than the old strain at low temperatures. The last statement is illustrated in Figure 18, where the estimated developments (by the linear equation) were prolonged until crossing the y axes, so that the lower temperature thresholds (where lines crossed the x axes) are presented per strain. No statistical differences were observed in survivorship of the two strains developing in constant temperatures (Figure 19).

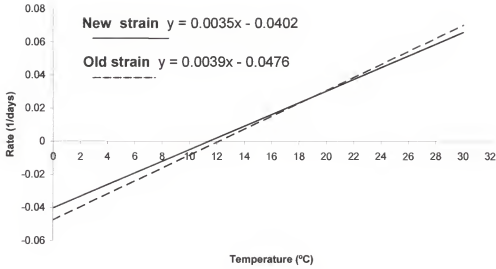

Figure 18. Linear development estimates by linear regression equations of the two strains of *Ormia depleta* at constant temperatures. Lower development was prolonged until crossing "y" axis.

The lower temperature thresholds for the two strains were compared following the procedures in Infante and Gil (1984) and Sokal and Rohlf (1995), showing the results in Table 30, where the thresholds of the two strains overlapped at 95% confidence making them statistically not different. Nevertheless, it was the new strain that showed the lower temperature at the lower confidence limits of the two strains (Table 20 and 27 and Figure 18).

Table 30. Comparison of slopes (b1)[1] and intercepts (a)[1] between the "new" and "old" strains of *Ormia depleta* by t-test and confidence limits at constant temperatures.

	Slope[2]	CL1	CL2	Intercept	CL1 (LTT)	CL2 (LTT)
New strain	0.00352 a	0.0034	0.0036	-0.0402 a	-0.0380 (10.78)	-0.0424 (12.04)
Old strain	0.00391 b	0.0037	0.0041	-0.0476 a	-0.0432 (11.05)	-0.0520 (13.28)

[1] b1 and a are the coefficients from the linear regression equations.

[2] Means followed by defferent letters are statistically significant.

CL1 = 95% upper confidence limits,

CL2 = 95% lower confidence limits.

LTT = lower temperature (°C) threshold

Male, female and total life cycles were compared between the new and old strains of *Ormia depleta* reared at constant temperatures. In all cases the comparisons showed statistical differences ($p < 0.0001$) (Figures 15,16 and 17). Based upon these results, it can be said that the two strains develop at different rates, which reinforces the previous results where developmental times were different at pupal and total life cycles.

These findings may support the hypothesis that the new strain is better adapted to develop at extreme temperatures than the old strain that failed to establish populations in north Florida. These results suggest the possible success of the new strain to establish populations north of 27° N latitude in Florida and neighboring states where the pest mole crickets are already established.

The previous information of life history of *Ormia depleta* has been refined to its relationship to constant temperatures, determining the ranges at which development occurs for the two strains released in Florida. Lower temperature thresholds per sex and pooled sexes were determined, along with a good approximation of what can be considered the upper temperature threshold for the two strains.

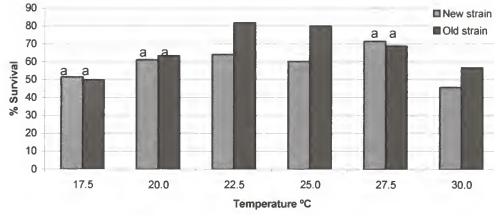

Figure 19. Survival (%) of the two strains of *Ormia depleta* at constant temperatures. Means followed by different letter are statistically different ("X^2 – test", $P<0.05$).

Development of the two strains of *Ormia depleta* under field conditions

Introduction

Studies of the tachinid endoparasitoid *Ormia depleta* have been devoted to its laboratory rearing techniques and seasonality of the adult stage. The only information available about the life cycle is under laboratory conditions. No study of this kind has ever made under field conditions.

Efficacy of this fly as biological control agent has been evaluated by direct and indirect means in Florida, but data so far obtained are sparse. Extensive data are needed to examine effect on host mole cricket populations, with geographical, seasonal, and annual variation.

This section describes the development of larval and pupal stages of *Ormia depleta* under field conditions at Gainesville, Florida (29°40'N). The two strains of this parasitoid were compared.

Materials and Methods

Gravid females of *Ormia depleta* new strain were obtained from the culture at the UF Entomology and Nematology Department, Gainesville, FL. Gravid females of *O. depleta* old strain were brought from the GCREC, Bradenton, FL, where they were captured in a trap using synthetic mole cricket song as bait. After capture at night, the flies were preserved alive by cooling them in a chest. The flies were still alive when they arrived in Gainesville.

Unless otherwise indicated, the host mole crickets were *Scapteriscus borellii* adults that were captured in traps baited with synthetic mole cricket song at the Ona REC, FL. The hosts were shipped in buckets partly filled with moist vermiculite.

Devices made of PVC pipe were designed to individually confine parasitized hosts underground at a range of depths. These pipe-chambers could easily be buried vertically and could be easily removed from the ground with little disturbance of mole cricket tunnels inside them. The pipe-chambers were subdivided into four sections with the assumption that each section represented a particular thermal regime and the total individuals confined at each depth can be considered as part of an independent experiment. Thus, it was possible to handle four independent experiments at the same time, covering different possible thermal regimes at which the host mole crickets naturally live and thus the parasitoids develop. The depths of the four chamber sections were: 0-15, 15-30, 30-45, and 45-60 cm (Table 6.)

The pipe-chambers consisted of 5 cm × 75 cm long PVC pipes that were cut lengthwise. A 2.5 cm wide × 25 cm long window was cut at the center of each half and sealed with aluminum window screen fixed with hot melted silicone. The inside of each pipe was subdivided into 15 cm sub-chambers, by placing non-corrosive metallic mesh, fully matching the inner diameter of the pipe once closed, also fixed with hot melted silicone. Starting from the bottom, the pipe-chambers were subdivided into four sub-chambers (15 cm tall × 5 cm diameter), leaving a portion of 15 cm intended to be above the ground level for handling purposes. This chamber design allows the host mole cricket to move freely in a volume of 300 ml of sand. The screened windows allow the sand inside the chambers to be in close contact with the sand outside, allowing the flux of air and moisture from the external soil into the chambers. The temperature was monitored inside and outside the chambers at the different depths, with no difference observed.

Moisture was not measured but was estimated by touch; tap water was added when considered necessary.

Each half chamber was filled with autoclaved moist sand, lightly compressed. Compressing the moist sand by finger made artificial tunnels. The halves of the chamber were joined as a sandwich by placing rubber bands at both extremes and at the middle. To check the status of host or parasitoid, or to feed the host, the pipes were withdrawn from the soil, the rubber bands were removed, and each half of the pipe-chamber was rolled gently to a concave position to avoid disturbance of the contents.

The land where the pipe-chambers were buried was kept free of weeds for the duration of the experiments. It was then assumed that the irradiation, aeration, and moisture (from environmental humidity and precipitation) were evenly distributed without interference from vegetation. The presence of patches of weeds would have modified the flux of these factors. A rectangle of land, 1 m wide × 6 m long, located near the Entomology and Nematology Department building, was tilled to 75 cm depth to allow the manipulation of the pipe-chambers in and out of the ground. Before placing the parasitized hosts into the chambers, these were buried underground for a few days. The chambers were symmetrically distributed, allotting five chambers to the width and 20 chambers to the length of the experimental land. Parasitized hosts were randomly allotted to the sub-chambers.

The parasitism of hosts was achieved following the procedure described by Wineriter and Walker (1990). Planidia were removed from gravid flies and 3 planidia were placed on the soft connective tissue behind and underneath the posterior of a host pronotum. Only mature planidia were used, recognized by their dark color. Immature

(light colored) planidia were discarded. A few minutes after they were parasitized, the hosts were placed in moist sand inside the pipe-chambers and buried underground.

The parasitized hosts were fed twice a week with the same Purina cricket chow food in use in the laboratory culture. Tap water was added every time the sand in the chambers was noted as dry to prevent hosts dying of dehydration.

The newly formed pupae were kept unmanaged the first five days, then they were transferred to a small container made of 2.5 cm diameter and 5 cm long transparent plastic tube with ends capped with fiberglass window screen tightened with small rubber bands. The small containers were half-filled with the same sand where the pupation occurred. The pupae were lightly covered with sand. The empty space in the small container allowed the newly emerged flies to crawl once emerged, and sometimes even to expand their wings. After the transfer to the small containers was made, the pupae were observed every six hours until adults emerged. The unopened pupae were kept under observation up to 15 days in the same place after the last adult emergence observed. Then the remaining pupae were taken to the laboratory to determine whether they were still alive, by dissecting them. Newly emerged adults were sexed. Surviving adult hosts were kept in the same sub-chamber for as long as 30 days more or until their death.

The soil temperature, at which the parasitized host was confined in the sub-chambers, was recorded by placing a thermocouple in the middle of the corresponding sub-chamber. Temperatures were recorded as follows using one pipe-chamber buried in the center of the experimental land. Four thermocouples were used, one at the depth of each of the four pipe sub-chambers. The thermocouples were connected to a digital temperature datalogger (HOBO® Mod H8-4, Onset Co.) programmed to record

temperatures every 30 min. The temperature recorded was downloaded to a computer for further analysis. Another digital temperature datalogger was used to record air temperature, with the thermocouple at 2 m height above ground under a small shed roof.

The response variables were the time to develop for larval and pupal stages. The observation frequency was every six hours, starting the fifth day after parasitism until the last cricket died because of the parasitic larval development. Pupal development was monitored every six hours starting five days after the fully formed pupa was observed, ending when the fly adult emerged.

The development of the two strains of the parasitic fly *Ormia depleta* parasitizing *Scapteriscus* mole cricket adults was evaluated at field varying temperatures through several experiments. The experiments were coded by the letter 'F' followed by the corresponding experiment number to track the period of time when each experiment was performed (Table 31). Then, a coded letter was added, corresponding to the sub-chamber depth and midpoint where the thermocouple was placed following the same nomenclature shown in Table 6 (p. 45). (i.e., F1-A = field experiment number one, which corresponds to the most superficial chamber of 0–15 cm depth, with temperature recorded at its midpoint at 7.5 cm). The data are presented by strain.

Table 31. Time periods when experiments were conducted for studying the development of the two strains of *Ormia depleta* at field-varying temperatures of Gainesville, FL.

Experiment codes	Period from parasitism to adult emergence (days)
New strain	
F1	May-June, 2000
F2	July, 2000
F3	August – September, 2000
F4	September – October, 2000
F5	October – November, 2000
F6	March – April, 2001
F7	April – May, 2001
Old strain	
F1	April – May, 2001
F2	April – May, 2001

The new strain

The development of the new strain was studied through seven experiments during 2000 and 2001, using the first two sub-chambers, A and B. *Scapteriscus borellii* was the only mole cricket used. The experimental design was random for all the experiments

The old strain

Two field experiments were run during April-May 2001. The first experiment used all four sub-chambers and two mole cricket species, *S. borellii* and *S. vicinus*; the experimental design was a factorial 2 × 4 with 10 repetitions. The second experiment used the two more superficial sub-chambers and one host species, *S. borellii.*

Results

The new strain

The results are shown in Tables 32 and 33, where it can be seen that the pupal stage takes longer to develop than larval stage. The effect of the seasonal temperatures is better noted in Figures 22 and 23, particularly for the influence of the maximum temperatures recorded during the development of the parasitoid by date.

In the superficial sub-chambers (0-15 cm depth) larval development ranged from 6.65 d (summer) to 10.12 d (fall); pupal development ranged from 9.0 d (summer) to 13.09 d (fall); total development ranged from 15.92 d (summer) to 28.07d (spring). In the second chambers (15-30 cm depth) larval development ranged from 6.92 d (summer) to 9.97 d (spring); pupal development ranged from 9.25 d (summer) to 12.54 d (fall); total development ranged from 16.17 d (summer) to 28.76 d (spring). The F-test showed statistical significance among stages and total development (Tables 32 and 33).

Table 32. Development time in days[1] and corresponding temperatures during development per stage life cycle of *Ormia depleta*, new strain, underground in sub-chamber A (0-15 cm depth) in field experiments.

Experiment	Stages			Temperatures ºC		
Code	Larva	Pupa	Total	Mean	Minimum	Maximum
F1-A	8.12 ± 0.15 c	10.17 ± 0.24 d	18.29 ± 0.28 f	28.1	24.3	36.6
F2-A	8.15 ± 0.10 c	9.00 ± 1.08 e	15.92 ±1.08 g	29.1	23.6	41.1
F3-A	8.30 ± 0.41 c	10.77 ± 0.59 c	19.07 ± 0.76 de	29.1	22.6	39.7
F4-A	6.65 ± 0.39 d	12.50 ± 0.32 b	19.14 ± 0.56 d	26.3	16.0	37.8
F5-A	10.12 ± 0.37 a	13.09 ± 0.43 a	23.21 ± 0.51 b	22.7	16.0	28.9
F6-A	LD	LD	28.07 ± 2.56 a	22.9	9.8	35.7
F7-A	9.77 ± 0.15 b	10.97 ± 0.26 c	20.74 ± 0.28 c	24.7	15.2	36.6

[1]Means (±SEM), followed by different letters are statistically different. Analysis by stage. LD = Lost data.

Table 33. Development time in days[1] and corresponding temperatures during development per stage life cycle of *Ormia depleta*, new strain, underground in sub-chamber B (15-30 cm depth) in field experiments

Experiment	Stages			Temperatures ºC		
Code	Larva	Pupa	Total	Mean	Minimum	Maximum
F1-B	7.57 ± 0.22 c	9.44 ± 0.33 e	17.01 ± 0.33 ef	28.5	19.1	39.1
F2-B	7.46 ± 0.37 c	9.25 ± 0.00 ef	16.17 ± 0.62 g	29.0	25.6	34.4
F3-B	7.66 ± 0.09 c	10.06 ± 0.12 d	17.73 ± 0.18 d	29.2	24.8	34.0
F4-B	6.34 ± 0.22 d	11.26 ± 0.30 b	17.60 ± 0.45 de	25.6	19.6	32.4
F5-B	9.97 ± 0.23 a	12.54 ± 1.40 a	22.51 ± 1.53 b	22.8	16.0	28.9
F6-B	LD	LD	28.76 ± 1.49 a	22.5	13.7	29.1
F7-B	9.79 ± 0.36 ab	11.23 ± 0.37 bc	21.01 ± 0.49 c	24.5	19.0	30.7

[1] Means (±SEM), followed by different letters are statistically different. Analysis by stage. LD = Lost data.

Although the mean temperatures were very similar during the experiments F2 and F3 at the two depths, the two stages and total development were statistically different. Something similar occurred with experiments F5 and F6, where the total development of the parasitoid was statistically different (Figure 20). Studies of the effect of fluctuating temperatures on insect development often show that development is more rapid at greater diurnal fluctuations (Worner 1992, Hagstrum and Milliken 1991).

The old strain

The F- test showed no statistical difference between hosts based upon which it was decided to consider all units as a single host (no distinction of host species). The mean developmental times and corresponding mean temperatures recorded during their development per depth are shown in Table 34.

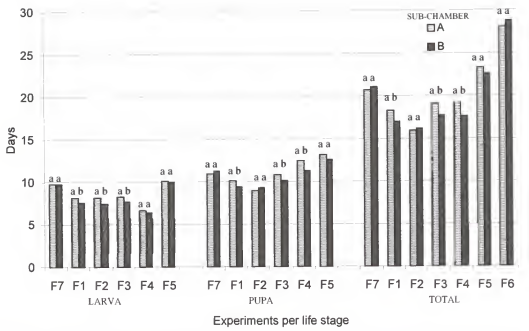

Figure 20. Comparison of larval, pupal and total development of *Ormia depleta*, new strain, at two depths at field (F) experiments. Means followed by different letters are statistically different. Analysis by depth.

Table 34. Development time in days[1], standard errors and corresponding temperatures during development per stage life cycle of *Ormia depleta,* old strain, underground at various depths in field experiments.

Experiment	Stages			Temperatures ºC		
Code	Larva	Pupa	Total	Mean	Minimum	Maximum
F1- A	8.55 ± 0.1 a	13.45 ± 0.1 a	22.00 ± 0.0 a	24.5	15.2	33.6
F1- B	8.44 ± 0.2 a	12.92 ± 0.1 a	21.36 ± 0.3 a	24.6	20.2	28.3
F1-C	8.90 ± 0.2 a	12.69 ± 0.1 a	21.60 ± 0.3 a	23.9	21.7	26.3
F1-D	7.92 ± 0.3 ab	13.55 ± 0.3 a	21.47 ± 0.1 a	23.5	22.1	24.8
F2- A	8.00 ± 0.0 c	12.90 ± 0.1 a	20.90 ± 0.1 b	23.7	15.2	33.6
F2- B	8.12 ± 0.1 bc	13.13 ± 0.2 a	21.25 ± 0.2 ab	23.8	19.0	27.5

[1]Means (±SEM), followed by different letters are statistically different. Analysis by stage.

The mean development times at the several depths by field experiments were not different, according to Duncan's multiple range test, Figure 21. This is not very surprising considering that during the spring, the temperatures were less variable, remaining rather cool and without big differences in mean soil temperatures (maximum

difference was 1.1°C). In the F1 experiment can be observed how variability in temperature decreases with depth in the soil, Table 34.

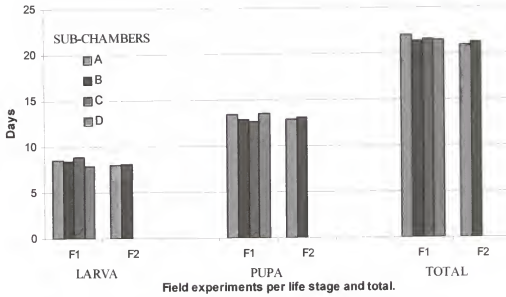

Figure 21. Comparison of larval, pupal and total development of *Ormia depleta*, old strain, at two depths in field (F) experiments. Means by experiment are statistically not different: Duncan's test.

Development times

The development time fluctuated according to the temperatures, and temperatures varied according to the time of the year. This relationship makes it difficult to compare the development times between strains and between experiments. However, temperatures in spring (experiments F6 and F7 with the new strain, and F1 and F2 with the old strain) were the least variable. The mean temperature recorded during experiment F5 (Oct.-Nov.) with the new strain was very similar to those aforementioned. All these experiments (four data sets for larval and pupal development and five data sets for total development) were grouped to run F-tests and mean comparisons between experiments and fly strains at the same depth (Figure 22).

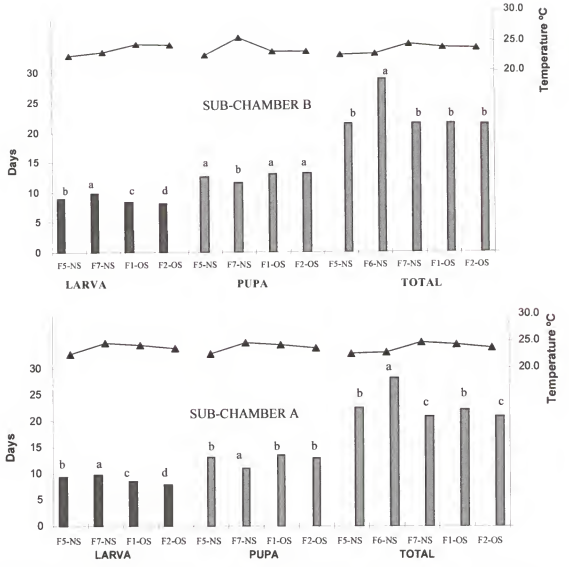

Figure 22. Comparison of larval, pupal and total developments of the two strains of *Ormia depleta* at two depths underground. Field experiments (F) selected by similar mean temperatures. NS = new strain, OS = old strain. Means followed by different letters are statistically different. Mean comparisons grouped by stage and total.

Larval development time was evaluated from two experiments per strain (thus 4 data sets), where all four sets were statistically different in each of the two. Pupal development times were in general not different from each other, but experiment F7 with the new strain, where the highest temperatures occurred (relative to the temperature range of all the experiments considered in this comparison), resulted in the shortest

development time, statistically different. Total development time in F6 (new strain) was longer than in all other experiments, coinciding with the lowest temperatures recorded. The differences were not very clear between total developments of the two strains in chambers A and B.

Discussion

Development times

The development of larval and pupal stages and thus the total development of the two strains of *Ormia depleta* were evaluated under field conditions. Seven field experiments at two depths were performed with the new strain, and two field experiments, at four depths in one experiment and two depths at the other, with the old strain. The whole set of experiments was accomplished by use of 22 parasitoid cohorts (= 22 experiments). A comparison of the development of the two strains showed no clear differences between them when the temperature regimes were most similar.

The mean developmental times of the new strain recorded at the two depths show little significant difference, and in most of the cases there was no difference between the two depths evaluated (Figure 20). The mean developmental times of the old strain recorded at the four depths show few significant differences.

The differences observed in development times of the new strain occurred during the highest temperatures recorded in the year, with the direct effect of stronger irradiation that where absorbed and transmitted through the bare soil in the experimental land, primarily affects the most superficial sub-chamber. Apparently, the depth of soil around the deepest sub-chambers, perhaps aided by moisture content of the soil, reduced the effect of that stronger solar irradiation during most of the year, which might partly explain the lack of differences between the deepest sub-chambers. Presence of vegetation

might add further insulation, so the development of this parasitoid might be expected to fluctuate less under vegetated soil where temperature and moisture would be more stable.

Problems

It is important to mention that the most important problem faced during the experiments with the new strain was caused by two fire ant species, *Solenopsis invicta* Buren and *Diplorhopium* sp. The huge mobilization capability and aggression of *S. invicta* inflicted severe losses on the host mole crickets, thus affecting the parasitic larval development. The small fire ant *Diplorhopium* sp. was more frequently seen devouring the newly emerging fly adults, causing the loss of data on sex of the newly emerged flies. Most of the cases when the small fire ant attacked a still living mole cricket, which was weakened due to the parasitic effect, the parasitic larvae were able to pupate inside the body of the dying mole cricket. In very few cases the same behavior was observed when *S. invicta* attacked. Very often the parasitoid pupation happened very close to the mole cricket carcass, which can be an important source of moisture for the developing pupae.

F6 was the experiment most severely affected by a nocturnal *S. invicta* invasion, forcing me to remove the chambers to another side of the experimental land, but not before losing most of the hosts and developing larvae. To avoid problems with ants in future experiments, it would be advisable to try covering the lateral windows with a fine metallic mesh able to prevent the small fire ants from entering the chambers but allowing the temperature, moisture and aeration flux into the chambers.

The other major problem was with the temperature-recording datalogger. It stopped several times during experiments F1, F3, F4 and F5, losing temperature data for some period of time in each of these experiments. The lost temperatures were estimated

by means of modeling portions of my temperature records using the Florida Automated Weather Network (FAWN) data from a station in Gainesville at a very short distance from the experimental land. A stepwise linear regression method was applied. It used 500 of my data points as dependent variables, and the air and soil temperature records from FAWN as independent variables. The procedural details of the temperature model are presented in Appendix C.

Diapause

Wineriter and Walker (1990) speculated that *Ormia depleta* may overwinter as a pupa, and may diapause. However, the experiments reported here gave no indication of diapause, and some pupae developed during November when on some days, for short periods of time, the temperature in the more superficial chambers dropped to 14°C. The experiments reported here were not intended to investigate the possibility of diapause and they do not rule it out. First, some of the mole crickets overwinter as adults, after having mated late in the year, resuming reproduction next year, thus suspending reproduction during the coldest months. Suspended development of *Ormia depleta* planidia might conceivably occur inside such mole cricket hosts due to factors other than temperature. Second, experiments were not conducted during the coldest months, the very time when diapause would be likely to occur.

Degree-day model predicting the development of *Ormia depleta.*

Introduction

Because temperature affects the development rate, fecundity, and mortality (and therefore the dynamics) of insect populations, considerable research on insect development in relation to temperature has been done. The purpose of many studies was to determine an appropriate development rate function for a phenological or general population model that can be used to predict important events in insect life cycles, or insect abundance, for control purposes (Candy 1991, Worner 1992, Lactin et al. 1995), which has been extended to estimating the effect of parasitoids on host populations (Gould and Elkinton 1990).

The rate of insect development depends upon the temperature to which the insects are exposed. For a developing insect, the rate at which time passes depends on the combination of its temperature-dependent development rate curve and the temperature at which it lives. The development rate curve may be altered by many factors of the environment, such as humidity, nutrition, or disease, but the curve always remains (Taylor 1981, Vinson and Iwantsch 1980). For each species, the temperature below which no measurable development occurs is its threshold of development. The amount of heat required over time for an insect to complete some aspect of development is considered to be a thermal constant (Campbell et al. 1974).

Scapteriscus mole crickets are pests of pasture and turf grasses and vegetables in the southeast and are spreading westward in the USA. They are soil-inhabiting insects, reaching the ground surface for dispersal and mate finding. Their life cycles are described in Chapter 2.

Ormia depleta is a South American tachinid that attacks mole crickets of the genus *Scapteriscus* (Fowler 1987). Adults are crepuscular and nocturnal parasitoids. Females are phonotactic, locating their hosts by their calling songs, and they deposit larvae on or near the host (Fowler and Kochalka 1985). Its life cycle is described in Chapter 2.

Two strains of the larviparous endoparasitic *Ormia depleta* have been released in the USA. The first strain (old strain) released in Florida established populations in central and south Florida, failing to survive in the northern part of this state, perhaps in some way due to low winter temperatures (Frank and Parkman 1999). A second strain (new strain) has been released in Georgia and North Carolina, and it may be released in north Florida, with the hope that it will tolerate winter temperatures and establish populations. There would, however, be no point in making further releases of the new strain unless evidence can be obtained that it is somehow more tolerant of colder winter temperatures. The life cycle duration and some basic statistics of both fly strains at constant temperatures and all the data necessary to build a degree-day model of this parasitoid are presented in Tables 19-21. The life cycle of both strains developing underground in the field during all but the coldest months of the year is presented in Tables 26-28.

The objective of this work is to evaluate the degree-day model based upon constant temperatures to fit the field development of *Ormia depleta* parasitizing mole crickets in north Florida (29°40'N).

Materials and Methods

Ormia- parasitized mole crickets were held at several constant temperatures. The developmental time per *Ormia* individual is converted to developmental rate by the reciprocal of time. The rates are then regressed against their corresponding temperatures

per life cycle stage and total development. A degree-day model is built based upon regression parameters (Eq. 1). The total degree-days required to develop are calculated by the reciprocal of the slope (b_1), and the lower threshold is calculated by dividing the intercept by the slope and multiplying it by –1.

$Y = a + b(x)$: (Eq.1)

Y = Developmental rate (1/days)
a = Intercept
b = Slope
x = Temperature °C

Once having calculated the linear equation describing the development of the parasitoid at constant temperatures, the corresponding lower threshold per stage and total are estimated.

The degree-days required to complete development at field-varying temperatures are estimated by the addition of degree-day per unit of time at which the temperature is recorded per stage and total at various depths in each field experiment. The estimation of the degree-days is performed by equation 2:

$$Y = \sum_{t=1}^{n} T(t - t_0) \qquad \text{Eq. 2}$$

Y = Degree-days
T = Time (time step = 30 minutes = 1/48 days)
t = Temperature recorded every 30 min
t_0 = Lower temperature threshold (per corresponding stage or total)
n = Number of days for stage to develop
i = Number of individuals

The equation is applied to every temperature recorded during the development (recorded every 30 min) of every parasitic individual, and then the degree-days are averaged per cohort and plotted on the same graph together with the estimated degree-

days at constant temperatures. All available mean degree-days of individuals that developed at every depth were included, grouping the data per larval, pupal and total development per fly strain.

Results

The two strains of *Ormia depleta* completed development at constant temperatures ranging from 17 to 30°C. None of the larvae completed development under 17°C or above 30°C. Developmental times (days) were transformed to rates, then plotted against their respective temperatures (Figure 8, and Figure 15,), showing a linear development covering the full temperature range (17 to 30°C) for larval, pupal and total development.

The development of *O. depleta* at field-varying temperatures was recorded from 12 cohorts of the new strain, and from six cohorts of the old strain. All experiments included two soil depths; the old strain included four soil depths in one data set (see Table 6, p 57). The new strain field experiments covered all the seasons of the year but winter; the old strain experiments were run during spring only.

The new strain.

The linear equation that best estimates development of *O. depleta* (new strain) at constant temperatures, lower thresholds and degree-days are presented as a linear equation model in Table 35. These parameters were calculated from studies at constant temperatures (Table 2 p. 48).

Table 35. Parameter values for the linear model, lower thresholds and thermal constants (degree-days) for development of *Ormia depleta* (new strain) per stage and total at constant temperatures.

Stage	Linear equation[a] $Y = a + b(x)$	Lower threshold (°C) $t_0 = -(a / b)$	Thermal constant[c] $k = 1 / a$
Larva	$Y = -0.092 + 0.0082(x)$	11.22	123.41±0.90
Pupa	$Y = -0.071 + 0.0062(x)$	11.54	163.6±0.92
Total	$Y = -0.040 + 0.0035(x)$	11.40	286.60±1.3

Y = Developmental rate (Y = 1 / days), a = intercept, b = slope, x = temperature
t_0 = lower temperature threshold; k = Degree-days±SEM (days)

Table 36. Degree-days required by *Ormia depleta* (new strain) to develop in incubation chambers and at field-varying temperatures.

Condition	Larva		Pupa		Total	
	Temp.°C	DD±SEM[b]	Temp.°C	DD±SEM[b]	Temp.°C	DD±SEM[b]
Constant						
	17.0	136.34±7.2	17.0	166.59±2.2	17.0	302.93±5.3
	17.5	131.69±2.3	17.5	176.09±1.1	17.5	301.31±3.4
	20.0	124.68±1.5	20.0	170.52±1.4	20.0	295.31±1.6
	22.0	131.87±4.3	22.0	160.94±1.4	22.0	292.81±5.4
	22.5	115.59±2.1	22.5	158.48±1.2	22.5	273.73±3.1
	25.0	118.47±1.8	25.0	159.78±1.6	25.0	277.35±1.8
	27.5	114.24±1.4	27.5	158.27±1.1	27.5	279.29±3.1
	30.0	128.74±1.5	30.0	171.44±1.6	30.0	300.18±2.3
Field[a]						
F1 A	28.8	140.68±1.3	27.8	166.18±1.9	28.33	305.59±2.3
F1 B	29.3	136.7±1.9	27.8	154.41±2.5	28.51	290.61±2.6
F2 A	29.3	124.7±0.0	28.9	156.37±0.0	29.05	279.10±0.6
F2 B	29.2	124.1±0.0	28.9	160.87±0.0	29.00	288.10±0.0
F3 A	29.4	148.7±2.6	28.8	184.86±2.7	29.01	332.43±3.0
F3 B	29.1	137.1±0.8	29.3	178.4±0.6	29.23	316.03±1.4
F4 A	29.4	115.4±1.1	24.7	159.2±1.9	26.40	267.07±2.8
F4 B	27.6	122.2±2.7	25.6	163.9±1.6	26.34	284.84±2.9
F5 A	23.3	115.6±0.8	22.7	145.7±2.0	22.75	253.83±1.7
F5 B	24.3	116.3±1.1	23.5	154.6±6.5	23.65	269.91±6.9
F7 A	23.3	110.5±0.5	26.1	159.9±2.2	24.74	275.32±1.8
F7 B	23.3	119.5±2.7	26.6	157.8±2.0	24.54	271.58±2.5

[a]Codes per experiment (see Tables 6, p. 45 and 31, p. 88). Mean temperature during development; [b]Average degree-days calculated with Eq. 2, all observations included.

The lower threshold values were used in Eq. 2 to calculate the accumulated degree-days required for *O. depleta* stages to develop at the two rearing conditions. Table 36 shows the mean degree-days per fly cohort and corresponding mean temperatures at which the development occurred during all experiments. Mean temperatures ranged from 22.7 to 29.4ºC, which fell inside the optimal temperature range, estimated through the constant temperature experiments.

The complete data set, comprising all observed developed *O. depleta* new strain, along with individual temperature at which each individual developed are depicted in Figure 24. The dispersion of the data appears to show a positive trend with relation with temperature increases. The data regressed against corresponding temperature, including those constant temperatures comprising field development only (Table 37), show the slope values and statistical significance from regression analysis, demonstrating statistical significance of the slopes for all stages under both rearing conditions. The next step was to evaluate whether the slopes of both temperature conditions matched the estimate of the degree-days for development at field-varying temperature. The slope from constant temperatures was compared with the slope from field-varying temperatures for each stage; calculating the 95% confidence limits made the comparison (Table 38). All confidence limits overlapped between constant and field-varying temperatures, meaning that regression slopes from constant temperature experiments are statistically not different from slopes from field temperatures. The constant temperature data set can be used to predict development of this parasitoid at any period of time of the year but winter.

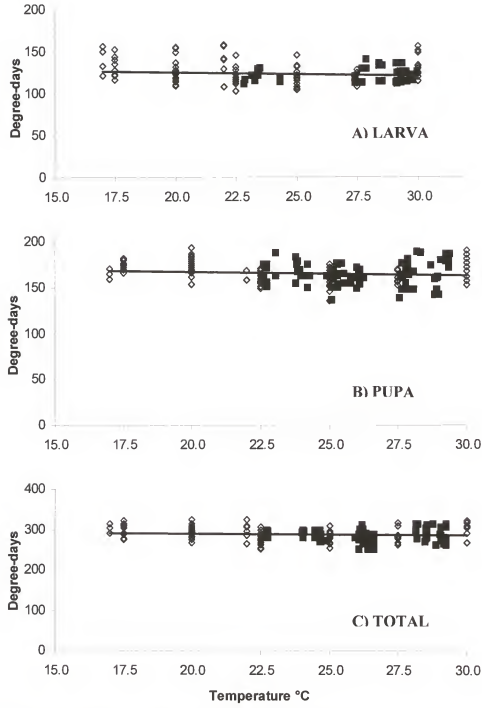

Figure 23. Regression lines of development of *Ormia depleta* (new strain) in incubation chambers set at constant temperatures (■Chambers ▬) and at field varying-temperatures (◆ Field). A) Larval development; B) Pupal development; C) Total development.

Table 37. Statistical significance of slopes in regression analysis of development of *Ormia depleta* (new strain) stages and total, in field and in incubation chambers.

Condition	Larva	Pupa	Total
Field[a]	2.97 (p= 0.001)	3.31 (p= 0.001)	8.77 (p= 0.01)
Chambers[b]	2.35 (p= 0.01)	3.01 (p= 0.01)	8.25 (p= 0.001)

[a]Development at field-varying temperatures (n = 156).
[b]Development in incubation chambers at constant temperatures (n = 135).
[c]Slope from regression pooling all observations (statistical "p" value inside parenthesis)

Table 38. Regression slope comparison by 95% confidence limits of degree-days of developmental stages of *Ormia depleta* (new strain) developing at field-varying temperatures and in incubation chambers at constant temperatures.

	Larva		Pupa		Total	
	UCL[a]	LCL[b]	UCL[a]	LCL[b]	UCL[a]	LCL[b]
Field[c]	2.28	3.65	2.58	4.04	7.65	9.90
Chambers[d]	1.85	2.86	2.37	3.66	7.31	9.19

[a]Upper confidence limits
[b]Lower confidence limits.
[c]All observations included (n = 156)
[d]Included: only constant temperatures in the range of field development (22.5 to 30°C) (n = 135).

The old Strain.

The linear equation that best estimates development of *O. depleta* (old strain) at constant temperatures, lower thresholds and degree-days are presented in Table 39. The equation coefficients were calculated from studies at constant temperatures (Tables 24 to 26).

Table 39. Parameter values for the linear model, lower thresholds and thermal constants (degree-days) for development of *Ormia depleta* (old strain) per stage and total at constant temperatures.

Stages	Linear equation[a] Y = a + b (x) (Eq. 1)	Lower treshold (°C)[b] t_0 = - a / b	Thermal constant[c] k = 1 / b
Larva	Y = -0.104 + 0.0090(x))	11.52	112.4±1.36
Pupa	Y = -0.087 + 0.0069(x)	12.61	146.1±1.26
Total (All stages)	Y = -0.048 + 0.0039(x)	12.20	258.2±2.14

Y = Developmental rate (Y = 1 / days), a = intercept, b = slope, x = temperature.
t_0 = lower temperature threshold
k = Degree-days±SEM.

The lower threshold values were used in Eq. 2 to calculate the accumulated degree-days required for *O. depleta* stages to develop under the two rearing conditions. Table 30 shows the mean degree-days per fly cohort and corresponding mean temperatures at which the development occurred during all experiments. Mean field temperatures ranged from 22.5 to 24.5°C, which fell inside the optimal temperature range, estimated through the constant temperature experiments. The narrow temperature range was because experiments with the old strain were performed during April and May, when soil temperatures remain rather low when compared with summer and fall months.

The complete data set, comprising all observed developed *O. depleta* new strain, along with the individual temperature at which each individual developed are depicted in Figure 29; although development at constant temperatures ranged from 17 to 30°C, Figure 29 shows those temperatures that cover field development temperatures only. The dispersion of the data appears to show a positive trend with relation with temperature increases. The data were regressed against corresponding temperature. Table 31 shows the slope values and statistical significance from regression analysis, demonstrating statistical significance of the slopes for all stages at both rearing conditions. The next step

was to evaluate whether the slopes of both temperature conditions matched the estimate of the degree-days to develop at field-varying temperature. The slope from constant temperatures was compared versus the slope from field-varying temperatures estimating development at the same stage; calculating the 95% confidence limits made the comparison (Table 32). All confidence limits overlapped between constant and field-varying temperatures, meaning that regression slopes from constant temperature experiments are statistically not different of slopes from field temperatures. The constant temperature data set can be used to predict development of this parasitoid at any period of time of the year but winter.

Table 40. Degree-days required by *Ormia depleta* (old strain) to develop in incubation chambers and in field-varying temperatures.

	Larva		Pupa		Total	
Condition	Temp.°C	DD±SEM[b]	Temp.°C	DD±SEM[b]	Temp.°C	DD±SEM[b]
Constant						
	17.5	115.03±3.3	17.5	149.94±1.9	17.5	249.33±2.4
	20.0	113.28±3.4	20.0	145.67±1.9	20.0	244.79±2.5
	22.5	104.37±1.4	22.5	140.16±1.8	22.5	244.60±1.9
	25.0	106.01±1.9	25.0	142.29±1.4	25.0	244.58±1.7
	27.5	115.74±2.9	27.5	151.69±1.1	27.5	247.94±2.3
	30.0	113.70±3.8	30.0	145.28±1.9	30.0	253.98±2.1
Field[a]						
F1 A	24.3	111.3±3.1	23.0	144.87±1.6	23.7	253.46±2.4
F1 B	24.5	110.5±1.9	23.8	135.05±2.5	23.7	249.83±1.3
F1 C	23.9	109.1±0.0	22.9	138.76±3.0	23.3	240.62±1.1
F1 D	23.5	95.2±0.0	28.9	136.87±0.0	23.1	234.41±3.2
F2 A	24.4	103.5±0.2	23.2	184.86±0.9	23.6	239.81±1.3
F2 B	24.5	105.1±0.8	23.4	142.4±2.3	23.8	247.44±2.2

[a]Codes per experiment (see Table 6, p. 59 and Table 31, p. 92). Mean temperature during development completion.

[b]Average degree-days calculated with Eq. 2, all observations included.

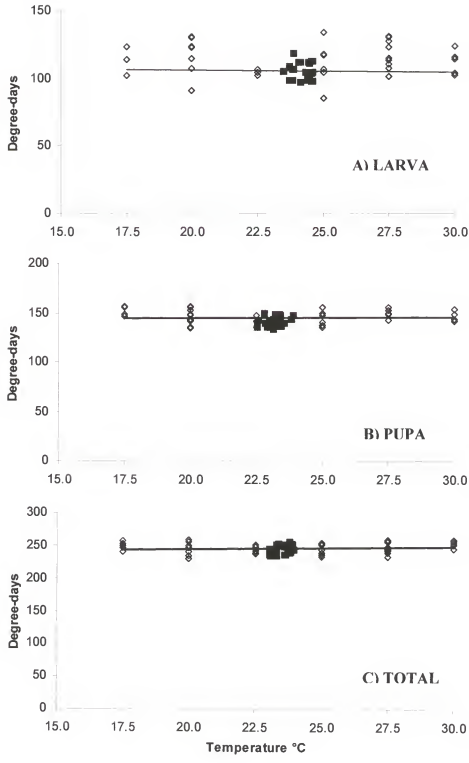

Figure 24. Regression lines of development of *Ormia depleta* (old strain) in incubation chambers set at constant temperatures (■ Chambers —) and at field varying-temperatures (◆ Field). A) Larval development; B) Pupal development; C) Total development.

Table 41. Statistical significance of slopes in regression analysis of development of *Ormia depleta* (old strain) stages and total, in the field and in incubation chambers.

Condition	Larva	Pupa	Total
Field[a]	4.09 (p= 0.001)	6.23 (p= 0.001)	14.99 (p= 0.001)
Chambers[b]	4.36 (p= 0.001)	5.33 (p= 0.001)	14.66 (p= 0.001)

[a]Development at field-varying temperatures (n = 85)
[b]Development in incubation chambers at constant temperatures (n = 33)
[c]Slope from regression pooling all observations (statistical "p" value inside parentheses).
ns = not significant

Table 42. Regression slope test by 95% confident limits of degree-days of larval, pupal and total development of *Ormia depleta* (old strain) in field-varying temperatures and in incubation chambers at constant temperatures.

	Larva		Pupa		Total	
	UCL[a]	LCL[b]	UCL[a]	LCL[b]	UCL[a]	LCL[b]
Field[c]	0.254	7.94	3.489	10.816	9.653	20.342
Chambers[d]	2.147	6.57	2.147	6.574	10.48	18.929

[a]Upper confidence limits
[b]Lower confidence limits.
[c]All observations included (n = 85)
[d]Included constant temperatures ranging field development (22.5 to 30°C) (n= 33).

Discussion

Development of eighteen cohorts of *Ormia depleta* (two strains) parasitizing mole crickets was studied at field-varying temperatures. The larval, pupal and total development degree-day model based upon experiments at constant temperatures proved good predictors of development at field conditions.

Ormia depleta development estimated at constant temperatures and evaluated as degree-days was a good estimator of development at field-varying temperatures. The coincidence of the constant temperature degree-day trend with observed development in

the field (Figures 24 and 25), is the first clue to the efficiency of constant temperature development to predict field development

The significance of slopes in regression analysis under the two conditions studied suggests that the degree-days required for *O. depleta* increase with temperature increases, although the thermal constant applies at the temperature range established in this study (Table 28).

Greater variability was observed among data obtained using the new strain than the old strain. This is explained by the fact that experiments with the new strain were performed at a wide range of naturally occurring temperatures in Gainesville, FL (29°40'N).

The data on the old strain were less variable, due to the narrow temperature range experienced in the field. Although the degree-days development from constant temperatures fitted development in the field, much more work will be necessary to determine the developmental response of the old strain during the year.

The results with the new strain are more relevant, not only because they are more complete, but also because this strain has been released in several states north of Florida. The phenological model developed in this work may provide the opportunity to understand and predict development of this parasitoid during the months when mole crickets are active.

One of the uses of the results presented in this paper is the ability to predict the period of time when this parasitoid would be active in relation to the host mole cricket calling seasons (flying and mating seasons) in north Florida. This knowledge will allow biologists and extension service personnel to use soil temperatures to when adult flies

will emerge from the soil and become active. Thus, the phenological model proposed in this paper can be integrated with the pest management of mole crickets. An example of a direct benefit of this phenological model would be the timing of pesticide applications according to the predicted fly season.

The use of the model is restricted to soil temperatures ranging from 17.0 to 30°C, which occurs from April to November or early December. This is the normal fly season. The soil temperature source can be the Florida Automated Weather Network, whose records proved highly coincident with soil temperature in the experimental area (at 22.5 cm depth, see appendix C) where development of the fly was studied.

CHAPTER 5
CONCLUSIONS

The objective of this dissertation was to describe the development of two insect parasitoids of mole crickets, the wasp *Larra bicolor* and the fly *Ormia depleta,* under a range of constant temperatures in the laboratory and at field-varying temperatures. The stages of work for each species were (a) experimental design, (b) conduct of the experiments and collection of data, (c) analysis of data, (d) fitting of the data to an accumulated degree-day model, and (e) validation of the models. In addition, although this was not included in the original plan, levels of parasitism of mole crickets by *Larra bicolor* were measured in two field populations in the autumn of each of two years.

The Parasitic Wasp *Larra bicolor*

1. The population reproductive statistics, such as fecundity, fertility and female longevity, of the progeny of wasps collected from the field during summer and autumn were statistically not different. Reproductive parameters such as net reproductive rate, intrinsic rate of increase, and generational time, were statistically different. These results indicate that the season of the year, defined by lower temperatures and shorter photo-phase during autumn, causes the wasp to modify the rates of development and reproduction. The statistical difference between seasons is explained by the fact that this parasitoid is adapted to modify its metabolism to overwinter as a pupa, which is reflected by doubling of the generational time in the progeny of wasps collected in the autumn.

2. Determination of development times of *Larra bicolor* at seven constant temperatures permitted an estimation of the lower developmental thresholds for egg, larva, prepupa, pupa, and total development. A precise upper developmental threshold could not be established with these data. However, the results indicate that the upper developmental threshold is near 32.5°C. The maximum survivorship of total development (from egg to adult) observed at constant temperatures was observed at 30°C, the minimum at 32.5°C, which supports the proposed upper threshold.
3. The development of the wasp *Larra bicolor* was studied under field conditions of Gainesville, FL. No statistical differences in development were observed when tracking the wasp developing at several underground depths. Statistical differences were observed among wasp cohorts that started developing in summer and autumn. Instead of the data showing a continuum from short to long developmental times, there was a trimodal pattern, with short (56.6 d), intermediate (112.9 d), and long developmental time (238.5 d), respectively. The long development that may occur from summer populations, more commonly from autumnal wasps, is considered to be the diapausing generation that provides the wasp populations the opportunity to survive cool winters and adult food shortage. This long development appears to be synchronized with the blooming of a plant that provides an important nectar source. This plant starts blooming in early May at this latitude (29°40'N). The overall survivorship of the wasp, from egg to adult, at field-varying temperatures was 13-14%; 58% of the wasps reached the prepupal stage, which indicates that the host mole cricket was devoured by the wasp grub. The overall sex ratio observed was 1:1.

4. Calculation was made of the degree-days required by stage and total development of *Larra bicolor* at constant temperatures. The linear models thus derived were applied to calculate the development of the wasp at field-varying temperatures. The accumulated degree-days required for wasps developing in the field were the same or very similar to those recorded at constant temperatures for the eggs, larvae, and prepupae. However, the pupal stage at constant temperature had only a short developmental time. Thus, the development times of pupae in the field, and the total development times (all successive immature stages) were underestimated for those individuals having pupae that took an intermediate time or a long time to develop.
5. Adult wasps of the strain that was released in north Florida, were observed parasitizing mole crickets. On average, 23% of the mole crickets captured in pitfall traps throughout the autumn of each of two years at two sites near Gainesville, FL (29° 40' N), were found to be parasitized by wasp eggs or larvae. Generational mortality inflicted on mole crickets by the wasp is obviously much higher. There was no statistical difference between parasitized mole crickets by sex, size, and developmental stages. Nymphs of small-medium size were observed being parasitized and some of these succeeded in producing adult wasps. Based upon these findings, the parasitic wasp can be considered to play an important role as a biological agent, reducing mole cricket populations in north Florida.

The Parasitic Fly *Ormia depleta.*

1. The development of the two strains (Piracicaba and Osório) of the tachinid fly *Ormia depleta* was studied per sex, stage, and total development at constant temperatures. Both strains develop at temperatures between 17 to 30°C; developmental time was

inversely related to temperature. Statistical differences were observed at pupal (thus at total development) stage between sexes, where males developed faster than females. Larvae developed faster than pupae at all temperatures. The survivorship of the strains differed, the Osório (new) strain showed maximum survivorship at 27ºC followed by 30ºC; the Piracicaba (old) strain showed maximum survivorship at 22.5º followed by 25ºC. Lower developmental thresholds were estimated followed by the degree-days required per stage and total by strain, to complete development. A precise upper developmental threshold could not be established with these data, however, the results indicate the upper developmental threshold to be near 32.5ºC, because no development of larvae and pupae occurred at this temperature. The new strain thus has a lower temperature threshold and it develops faster at lower temperatures than does the old strain. The old strain develops faster at the highest temperature (30.0ºC). These findings suggest that the new strain is better adapted to succeed at lower temperature regimes, and might be expected to establish a population farther north in the northern hemisphere.

2. Field evaluations were made, during April-November for the new strain, and during April-May for the old strain, at 29º40'N. The development time fluctuated according to the temperatures, and temperatures varied according to the season of the year. The differences were not very clear between total development times of the two strains developing during April-May 2001, thus exposed to very similar temperature regimes. The degree-days required for total development fluctuated from 250 to 330ºC-day, for all field experiments.

3. A degree-day model was constructed using the results from the study of *Ormia depleta* development at constant temperatures. The model was then evaluated for its goodness of fit to the field data. Field data fell into the predicted range.

Future Research

1. Suggestions for future research include evaluation of development times and survivorship for wasps and flies outdoors during the coldest months (December-March), and further evaluation of diapause. It is not known whether the flies can diapause. The observed trimodal pattern of development time of the wasp pupa is at least curious; it may be an artifact of too few observations, or it may be real. If it is real, then its control mechanism requires investigation.

APPENDIX A
DEVELOPMENT OF TWO MOLE CRICKETS, *S. VICINUS* AND *S. ABBREVIATUS*, AT CONSTANT TEMPERATURES.

Introduction

Three species of mole crickets (*Scapteriscus* spp.) are the most damaging turf and pasture grasses pests in much of the southeastern United States. They destroy turf and grasses by feeding on roots, leaves and shoots, and by tunneling, which disrupts the soil around roots, preventing uptake of water and nutrients (Walker 1985a, Hudson 1987). The pest mole cricket populations are spreading in the southern USA, damaging turf and pastures grasses.

Several authors (Hayslip 1943, Forrest 1985, Hudson 1987, and Braman 1993) studied the general biology, oviposition and development of mole cricket species. Those studies did not consider the effect of temperatures on the life cycle development of the mole crickets. Understanding the relationship between mole cricket life cycle and temperature would help interpretation of the seasonality of the pests and their interaction with the imported parasitoids in adapting to the pests' life cycle in Florida (see Chapter 2).

Having the description of the effect of temperatures on life cycle of mole crickets is the primary step toward modeling the mole cricket development that would enable the construction of models to understand the interrelationship of the organisms involved in the biological control of mole crickets. This work was aimed to determine the life cycle

development of two mole cricket species, *Scapteriscus vicinus* and *S. abbreviatus* at constant temperatures in incubation chambers.

Materials and Methods

Host mole cricket *Scapteriscus vicinus* adults were captured in traps baited with species-specific synthetic mole cricket song at Gainesville, FL (29°40'N). The hosts were transferred to the laboratory in buckets partly filled with moist sand. The *S. abbreviatus* mole crickets used in this research were obtained from the culture in the UF-Gainesville Entomology and Nematology Department.

The study of development of mole crickets requires keeping them in individual containers to prevent cannibalism, so this procedure was applied to *S. abbreviatus* in culture. Each mole cricket was confined in 300 ml containers, filled with autoclaved sand. The containers consisted of 7.5 cm diameter × 7.5 cm tall PVC-pipe with both ends capped with plastic petri dishes kept in place by rubber bands. The bottom of the containers was covered with black plastic to stimulate (by exclusion of light) the mole cricket female to oviposit, which facilitates the location and extraction of the eggs.

To start the experiment, it was necessary to be sure that all females were fertilized to produce progeny, starting ovogenesis at same time. The S. *abbreviatus* were all virgins, because in culture they developed in individual containers. Some of the *S. vicinus* (all captured from the field), may have already mated by the time they were collected (Walker and Nation 1982), but it was assumed that most of the females were virgins, or just recently mated; to promote uniformity, all the female crickets were collected during two days of the same week in early March, confining them immediately. Females were provided with males for a period of seven days, to ensure mating, then the males were

removed. The mid-point of the week was considered the date of mating, followed by ovogenesis.

Thirty two *S. vicinus* and 28 *S. abbreviatus* females were included in this work. The study was initiated the second week of March 1999, coinciding with the beginning of the *S. vicinus* activity season in Gainesville, FL; *S. abbreviatus* populations are not established at this northern latitude (29°40' N) (see Chapter 2).

The reproductive females were kept at a constant 26°C and photoperiod of 14:10 (D:L); the sand was kept wet by adding tap water when needed. The females were fed twice a week with the same mole cricket chow in use in the culture.

Oviposition, fecundity and fertility

The number of egg clutches per female and the periods of time between clutches were quantified. Thus, the oviposition period per mole cricket species was determined.

To determine when a clutch of eggs was laid, it was necessary to open the container and look for the eggs or for the oviposition chamber, causing minimal disturbance. The observations were made twice daily because, in previous experiments, *S. vicinus* was observed eating its own eggs even the same day they were oviposited. Although this was never observed to happen with *S. abbreviatus*, the observations were made at the same frequency, as a standard method. Sometimes a female may oviposit the total egg clutch in two steps. Usually spaced within a few hours, oviposition occasionally was spaced over to two or three days; when this happened, all eggs were considered to belong to a single egg clutch, but were labeled with distinct dates.

The same day the eggs were laid, they were confined in 2.5 cm plastic petri dishes containing a wet sponge wrapped with filter paper. The petri dishes were labeled with a

code for identification, date when oviposited, consecutive number of egg clutch, and number of eggs.

The egg clutches were randomly allotted to six environmental chambers, set at temperatures of 10, 15, 17, 20, 25, and 30°C, respectively. The proportion of eggs hatching was quantified per egg clutch. Then, the period of time required for the eggs to develop at each temperature was quantified. The observations of egg hatching were made daily, by looking at the petri dish containing the eggs, without opening it.

Nymphal development

As soon as hatchlings were observed, they were confined individually in 50 ml snap-capped plastic vials half filled with wet autoclaved sand; the vials were labeled, identifying them by code, date when where oviposited, hatching date, and consecutive number of nymph. Because nymphs usually eat their exuviae soon after molting, the hatchlings were marked with acrylic paint by placing a dot on their pronotum, allowing identification when they molted. Once molted, a new paint dot was placed, repeating it after every molt. When the adult stage was reached, it was sexed and marked for follow-up purposes. By this means, the number of nymphs and the period between nymphs per temperature were determined.

Results

Scapteriscus vicinus

Out of the 32 females put to mate, only 27 were selected, because two died and three devoured the male during the first two days, making it questionable whether they had mated.

Oviposition, fecundity and fertility

The ovipositions recorded by female (Table 43) showed that 57 egg clutches were oviposited by 27 *S. vicinus,* comprising 941 eggs that gave an average of 34.9 eggs/female during her lifetime. The average oviposition period estimated was as 61 days. The time estimated between clutches was 28.6 d.

The number of eggs per clutch was reduced with the number of clutches per female (Figure 25), producing the maximum number of eggs at the first egg clutch, then declining at the second clutch. The second, third and fourth egg clutches contained a similar number of eggs, but the fifth (last) contained fewer.

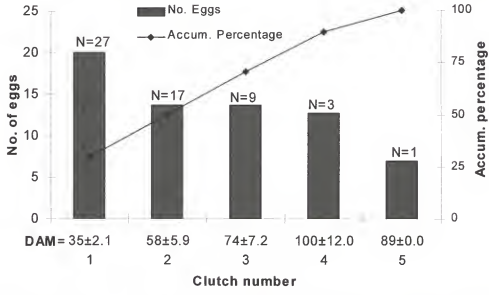

Figure 25. Number of eggs per clutch, time after mating, and accumulated percentage of eggs of *Scapteriscus vicinus* at 26ºC. DAM = Days after mating±SEM

The proportion of eggs that hatched per temperature is presented in Table 44, where is clear that the maximum egg survivorship occurred at 25ºC (22.7%) followed by 30ºC (16.5%) and 20ºC (8.6%). No eggs hatched at temperatures below 20ºC. In a

separate experiment I exposed some eggs and adults of *S. vicinus* to 35°C with no survivorship observed of either eggs or adults.

The time spent for egg development varied from 14.5 to 44.3 d, varying according to the temperature at which they were confined (Table 44). The unhatched eggs were kept two weeks at the same corresponding temperature, then transferred to a 26°C room and kept for two more weeks, and finally they were observed under the microscope to determine whether some development could be detected.

Table 43. Fertility and oviposition period of *Scapteriscus vicinus* reared at 26ºC and photoperiod of 14:10, L:D.

	MC[a]	1		2			3			4			5			Eggs	No.	Ovipos
n	CODE	E1[b]	T[c]	E2[b]	TO[d]	ATO[e]	E3[b]	TO[d]	ATO[e]	E4[b]	TO[d]	ATO[e]	E5[b]	TO[d]	ATO[e]	Total	Clutches	Period
v1	v1	11	10	13	15	25	13	13	38							37	3	38
v2	v2	32	10													32	1	10
v3	v3	21	25	21	56	81	23	12	93	14	27	120				79	4	120
v4	v4	25	26	16	11	37	10	37	74							51	3	74
v5	v5	14	37													14	1	37
v6	v6	22	38	12	24	62	9	14	76	11	3	79	7	10	89	61	5	89
v7	v7	14	43													14	1	43
v8	v8	11	49	12	30	79										23	2	79
v21	v9	30	10	9	33	43										39	2	43
v22	v10	26	10	21	18	28	20	11	39							87	3	39
v23	v11	29	12													29	1	12
v24	v12	28	12	5	92	104										33	2	104
v25	v13	22	13	13	7	24										35	2	24
v26	v14	27	17	11	14	31										38	2	31
v27	v15	23	17	5	35	72	3	14	86							31	3	86
v28	v16	3	19													3	1	19
v29	v17	9	34	11	48	82										20	2	82
v30	v18	25	38	26	26	64	21	23	87	13	17	100				85	4	100
v31	v19	26	25	24	17	42										50	2	42
v32	v20	16	41	15	12	53	19	20	73							50	3	73
v33	v21	28	54	6	31	84	4	12	96							38	3	96
v34	v22	28	57	12	9	66										40	2	66
v35	v23	32	59													32	1	59
v36	v24	6	66													6	1	66
v37	v25	5	68													5	1	68
v38	v26	9	73													9	1	73
v39	v27	20	74													20	1	74
	SUM															941	57	
	MEAN	20.1		13.6			13.6			12.7			7					
	MEAN		34.7		28.1	57.5		17.3	73.6		15.7	99.7		10	89	16.5	2.1	61.0

a. Female mole cricket code
b. Number of eggs per clutch.
c. Time in days after mating.
d. Time in days after previous clutch.
e. Accumulated time in days after mating.

Table 44. Egg fertility and development of *Scapteriscus vicinus* at constant temperatures.

Temperature ºC	Fertility % Hatching	Development Days
10	0	0
15	0	0
17	0	0
20	8.6 ± 5.82	44.25 ± 2.01
25	22.70 ± 7.09	19.31 ± 0.69
30	12.50 ± 4.89	14.50 ± 0.65

Development

The hatchlings obtained at each temperature were transferred into a small vial filled with wet sand and kept at the same temperature. Their development was tracked per individual. No egg development occurred at temperatures of 17°C or below; although some eggs hatched at 20°C, all hatchlings died at first instar. Therefore, nymphal development was studied at 25 and 30°C only.

Table 45 presents the developmental time of nymphs recorded from chambers at temperatures of 25 and 30°C. The hatchlings showed high susceptibility to 30°C, most dying after the first instar; only one individual developed until the ninth instar and then died without reaching the adult stage. At 25°C a few nymphs developed until the ninth instar and died. Vestigial wings were apparent from the 7th and 8th instars. The maximum development time recorded was 165 and 230 days at 30 and 25°C respectively.

Table 45. Nymphal developmental time of *Scapteriscus vicinus* at two constant temperatures.

	30°C			25°C		
Instars	n	Per instar	Accumulated	n	Per instar	Accumulated
1	13	17.43±0.29	17.43±0.29	28	20.21±0.74	20.21±0.74
2	1	30	47	17	16.88±2.11	38.73±2.21
3	1	LD	LD	11	18.27±2.37	54.27±5.11
4	1	19	66	9	23.33±3.18	77.77±7.38
5	1	19	85	8	22.38±0.94	104.25±7.49
6	1	28	113	7	20.57±0.72	121.29±7.91
7	1	18	131	5	22.40±2.77	140.83±10.76
8	1	25	156	4	40.50±4.97	177.40±17.68
9	1	8	164	2	40.50±20.5	230.50±27.5

Developmental times between instars ranged from 8 to 30 d at 30°C and from 18 to 40 d at 25°C. The development of the only nymph that developed at 30°C (Figure 26)

showed no consistent trend. At 25°C duration of the first seven instars was similar, then the last two instars, 7th and 8th, required a longer time to complete development (Figure 27).

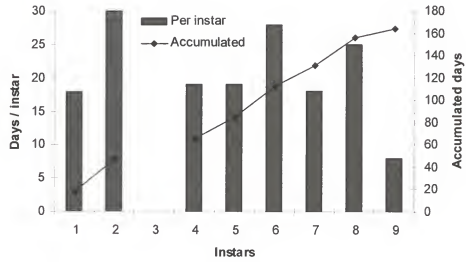

Figure 26. Nymphal developmental time of *Scapteriscus vicinus* at constant 30°C in an incubation chamber. N=13 for 1st instar; N=1 for the other instars.

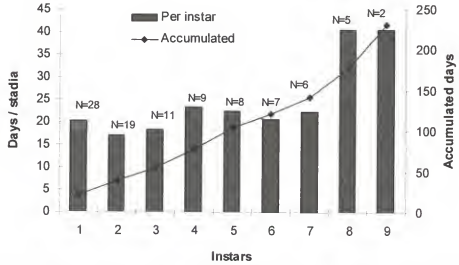

Figure 27. Nymphal developmental time of *Scapteriscus vicinus* at constant 25°C in an incubation chamber.

Scapteriscus abbreviatus

After the mating period, 25 females survived and all were included in the experiments. The males were taken out of the oviposition chambers, keeping the females in their respective chamber. The starting time of the oviposition time was the mid-point of the week when females mated.

Oviposition, fecundity and fertility

The mean oviposition period was as 101.2 d, ranging from 28 to 190 d with 21.7 days between clutches. The total number of clutches oviposited by the 25 females was 119, averaging 4.76 clutches per female. The total number of eggs recorded from the 25 females was 3918, giving a mean of 156.7 eggs per female during its lifetime (Table 46).

The average number of eggs produced per female per clutch is shown in Figure 28, where the mean number of eggs of the three first clutches comprised 50% of the eggs produced during each female's lifetime lifetime. The 50% were oviposited during the first 60 days after mating. The number of eggs per clutch declined with the clutch number (Figure 29).

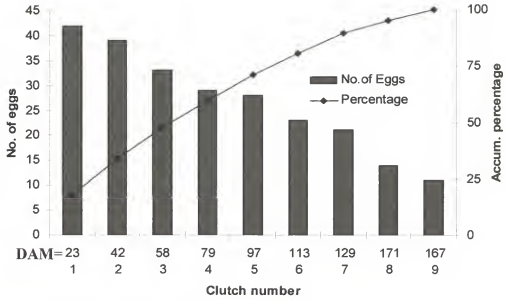

Figure 28. Number of eggs per clutch (average) and days between clutch and corresponding proportion (%) of eggs per time of *Scapteriscus abbreviatus* at constant 26°C. DAM= days after mating.

The proportion of eggs hatching was strongly related to the temperature at which they developed (Figure 30). The maximum egg survivorship was recorded at 25°C (51.26%), followed by 30°C (48.72%), and finally at 20°C (20.88%). No development occurred at 17°C or below. The unhatched eggs were treated as for S. *vicinus*.

The relationship between egg fertility and egg clutch number is not very clear (Figure 30), presenting strong variation from one egg clutch to the other with no significance among them. The only trend that was seen is that fertility of the first three clutches was higher than in the others.

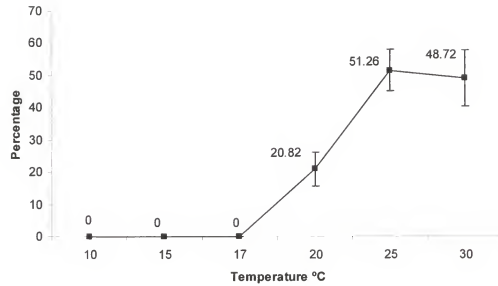

Figure 29. Proportion of eggs of *Scapteriscus abbreviatus* hatching at constant temperatures.

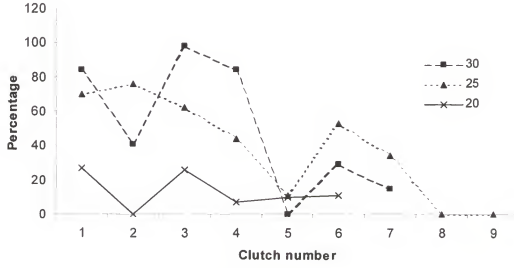

Figure 30. Proportion of eggs from *Scapteriscus abbreviatus* hatching by clutch number at constant temperatures.

Development

The hatchlings at each temperature were transferred into small vials filled with wet sand, confining them individually at the same temperature. No hatchlings from 20ºC survived the first instar. Therefore, larval development reported is from two temperatures, 25 and 30ºC (Table 46).

Table 46. Nymphal development of *Scapteriscus abbreviatus* at constant temperatures

	25ºC		30ºC	
Stadia	Per instar[1]	Accumulative[1]	Per instar[1]	Accumulative[1]
1	32.38 ± 0.60	32.39 ± 0.60	28.13 ± 1.03	28.13 ± 1.03
2	29.64 ± 2.46	50.41 ± 2.94	6.83 ± 1.72	39.56 ± 6.54
3	22.39 ± 1.33	63.88 ± 3.18	13.00 ± 3.59	76.26 ± 5.29
4	19.37 ± 1.31	79.04 ± 2.04	18.60 ± 1.69	102.20 ± 7.42
5	23.64 ± 1.24	102.50 ± 2.30	22.75 ± 2.35	121.00 ± 9.46
6	26.44 ± 2.10	136.17 ± 5.18	18.30 ± 1.93	140.60 ± 12.21
7	34.91 ± 4.33	172.83 ± 8.46	28.78 ± 4.93	175.20 ± 15.03
8	30.29 ± 5.19	164.17 ± 11.48	37.75 ± 6.52	171.50 ± 21.06
9	35.00 ± 0.00	189.00 ± 0.00	48.00 ± 0.00	191.50 ± 35.50

1. Mean±SEM

Nine instars were recorded during *S. abbreviatus* development. Time between instars ranged from 19 to 35 d at 25ºC and from 6 to 48 at 30ºC. The accumulated mean time required to pass through the nine instars at 25ºC was 189 d, and 191.5 d at 30ºC. The developmental times varied among the instars, the intermediate instars tending to develop in a shorter time than those at the extremes. This tendency was observed at the two temperatures evaluated (Figures 31 and 32). Fifty percent of the nymphal development was reached by the 5th instar at 25ºC and by the 3rd to 4th instars at 30ºC.

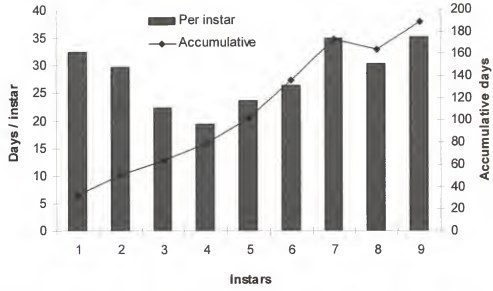

Figure 31. Nymphal development of *Scapteriscus abbreviatus* at constant 25°C.

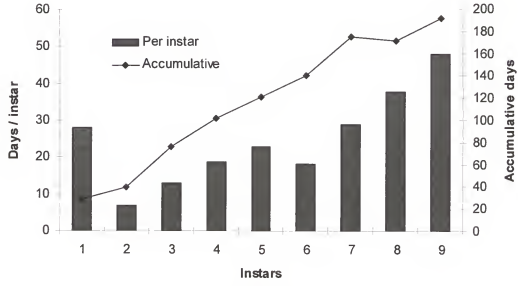

Figure 32. Nymphal development of *Scapteriscus abbreviatus* at constant 30°C.

The survivorship observed at the two temperatures was very low considering the total development from egg to adult. Thirteen individuals (out of 898 eggs; 498 hatchlings) reached adult stage at 25°C and 9 (out of 737 eggs; 384 hatchlings) at 30°C. The low adult proportion may have been due to the intense handling by applying the

paint dot and constant shaking of the nymphs when recording the molting time (observing the paint dot present or absent). Data from the few survivors (Table 47) show the time to develop from egg to adult, and sex ratio. The adult stage was reached from three instars, the 7th instar being the most common. The overall development at 25°C was 188.9 d and at 30°C was 202.7 d. The overall sex ratio was female-biased at 25°C and male-biased at 30°C

Table 47. Developmental time of *Scapteriscus abbreviatus* from egg to adult, grouped by number of instars to complete development and sex ratio per group.

	25°C			30°C		
Instar	No.	Time (d)[1]	Sex ratio[2]	No.	Time (d)[1]	Sex ratio[2]
7th	10	184.0±16.9	6:4	5	208.4±5.3	2:3
8th	2	213.5±30.5	2:0	2	199.5±6.5	0:2
9th	1	189.0±0.00	0:1	2	191.5±35.5	1:1
Overall	13	188.9±13.7	8:5	9	202.7±7.06	3:6

1. Mean days±SEM
2. Female: male

Discussion

Scapteriscus vicinus

S. vicinus females were able to oviposit up to 5 clutches with an overall average of 2.1 clutches per female with a mean number of 34.9 eggs per female. The most evident reduction in number of eggs per clutch occurred at the fifth clutch. The overall mean oviposition period was as 61 days. Forrest (1985) reported up to 10 egg clutches per female (without specifying mole cricket species, either *S.vicinus* or *S. borellii*) Braman (1993) reported up to 8 egg clutches for *S. vicinus* females and up to seven clutches for

S. borellii. Braman (1993) reported an average of 3.1 clutches per *S. vicinus* female with 45.8 eggs total per female and an average of 2.4 clutches with 58.2 eggs for *S. borellii* females. Meanwhile, Worsham and Reed (1912) cited in Braman (1993) reported a range of 29 to 60 eggs total per female S *vicinus*. Hayslip (1943) reported an oviposition period of 29 to 38 days.

There are several disagreements among the reports regarding the oviposition description of *S. vicinus*. Part of the cause of this variability might be the temperatures at which the studies were run. The results of the work presented here considered the oviposition process at constant temperatures in incubation chambers. Other authors studied the oviposition process at field temperatures, which may have been subject to the naturally fluctuating temperature regimes at varying latitudes.

The eggs of *S. vicinus* developed at temperatures ranged from 20 to 30ºC, failing to develop at lower temperature than 20ºC. Fecundity and fertility were strongly affected by the temperatures at which they were reared, with the maximum values at 25ºC, which is an indicator that this temperature is close to the optimum temperature for egg development. Braman (1993) reported egg development required 2-3 weeks at 27ºC, that is close to that observed in this work between 25 and 30ºC (19.3-14.5 d). Forrest (1985) reported that fertility decreases with egg clutch number; the later clutches a female produced had lower percent hatch which agrees with the observations of this work (Figures 31 and 32).

The mean number of eggs per female was as 16.1, ranging from 3 to 80 during its lifetime. The maximum fertility observed was 22.7%

Nymphal development was strongly affected by the ambient temperature. Development of more than one nymph was recorded only at 25ºC, showing that the immatures of this species are very susceptible to the temperatures at which they live.

Even though several nymphs reached the ninth instar, none of them reached the adult stage. Most of the 8th instars developed vestigial wings but fail to fully develop, which I attribute to the experimental conditions at which they were reared, because all the older nymphs died during molting. The possibility that the ninth instar nymphs were ready to transform to the adult stage is reinforced by Braman (1993) who observed individuals reaching adulthood at 8th and 9th instars. *Scapteriscus borellii* (=*acletus*) was reported to reach adulthood at 7th to 10th instars by Hudson (1987). *S. abbreviatus* also reached adulthood at 7th to 9th instars (see *S. abbreviatus* results in this account), indicating a standard range of instars for all three mole crickets to complete development to adulthood.

The total development of *Scapteriscus vicinus* from egg to the 9th nymphal instar was estimated as 250 days, considering egg development (19.4 d) at 26ºC plus nymphal development (238.5d) at 25ºC. Nevertheless, development time varies at temperatures between 20 to 30ºC. Hayslip (1943) reported (what he called "fall adult") nymphal development of 135 d plus 18 d of egg development (= 154 d total). Braman (1993) reported the nymphal development as 23 – 32.5 weeks (= 161-227 d). Hudson (1987) reported 223 d for total development (from egg to adult) for *S. borellii* (=*acletus*).

Advances in the study of the life cycle of *Scapteriscus vicinus* were made, establishing possible temperature boundaries for development, and more specific information was obtained for *S. vicinus* developing at 25ºC.

Scapteriscus abbreviatus

Results with this mole cricket species were more consistent, with several individuals reaching adulthood at the two temperatures where development occurred. There is no published information about general biology or life cycle development of this mole cricket species to compare. The lack of this information is explained by the fact that *S. abbreviatus* populations are restricted to a small area, close to the original areas where they arrived in the early 1900s. The lack of long wings prevents this species from dispersing readily.

Both species

S. abbreviatus is highly fecund, ovipositing more than twice the number of clutches as does *S. vicinus* (Tables 43 and 44) and the proportion of eggs hatched per clutch and per temperature was greater in *S. abbreviatus* than *S. vicinus (*Figures 33 and 34). The time to develop from egg to adult was shorter than in *S vicinus* (Tables 45 and 46).

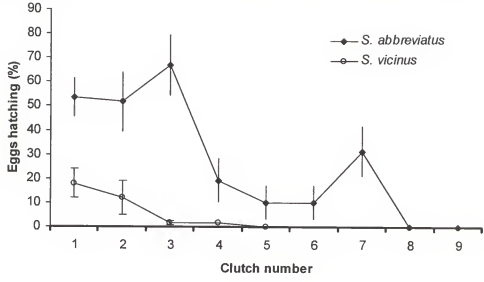

Figure 33. Number of eggs hatched per clutch of two mole cricket species, *Scapteriscus abbreviatus* and *S. vicinus* at constant 26°C.

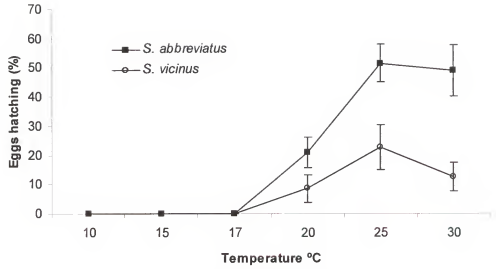

Figure 34. Proportion of eggs hatched of two mole crickets, *Scapteriscus abbreviatus* and *S. vicinus*, at constant temperatures.

S. abbreviatus developed as did *S. vicinus*, but with greater success in reaching the adult stage, perhaps due to this species being already in culture, and thus laboratory-adapted (at 26±1°C), or due to its higher fitness and reproductive capacity.

That fewer individuals reached adulthood at 30°C than 25°C, which coincides with the higher overall survivorship at the same temperature reinforces the possibility that 25°C is about optimal for *S. abbreviatus* development. Another clue supporting it is the lesser time to develop at 25°C than 30°C, which indicates the latter temperature surpasses the optimum, slowing down the development (requiring more time to complete development) (Wagner et al. 1984a).

That development of the two mole cricket species occurred at only a narrow range of temperatures makes modeling difficult, but it established temperature constraints. Future works should include intermediate temperatures between 20° and 30°C, and possibly some higher than 30° but less than 35°C.

Nymphal development of *S. abbreviatus* was well described and might give some clues to make culture more efficient. For example, the clutch number could be used to eliminate females once they have oviposited 75% of the expected oviposition total, saving time and money.

Modeling the egg development would enhance the probability of predicting successive nymphal events. For example, the time spent at 20ºC was 44 d which, coupled with the estimated 50% of flying females in the spring, helps to predict when nymphal stages will be present (something very hard to monitor in the field).

APPENDIX B
PARASITISM OF MOLE CRICKETS BY THE WASP *LARRA BICOLOR*

Introduction

Larra spp. wasps have been identified as specialist natural enemies of mole crickets in the tropics and subtropics (Adamson 1942, Castner 1983, 1988, Frank et al. 1995, Frank and Parkman 1999, Menke 1992, Williams 1928); they use particular species of mole crickets as hosts for individual ectoparasitic larvae; after 10–12 days mature larvae devour their weakened hosts (Castner 1988).

Species of *Larra* were used in the earliest attempts at classical biological control of mole crickets, in Hawaii in the 1920s and Puerto Rico in the 1930s (Frank et al. 1995). No analyses of their effect on mole cricket populations on these islands appear to have been published (Castner 1983, 1988, Frank 1994, Frank et al. 1995).

Adults of *L. bicolor* are specialist parasitoids of large nymphs and adults of pest mole crickets of the genus *Scapteriscus* (Castner 1984, 1988, Frank and Parkman 1999, Frank et al. 1995). In the early 1980s *L. bicolor* wasps were released at five Florida sites in conjunction with plots of their preferred nectar-source plant, *Spermacoce verticillata* L. (Frank et al. 1995). At that time only wasps released at one of the sites (in Fort Lauderdale) established viable populations (Frank et al. 1995). Castner (1988) reported 0% parasitism of *Scapteriscus vicinus* Scudder mole crickets (and <1% of *S. abbreviatus* Scudder) trapped at Fort Lauderdale during the period April 1985 through December 1987, leading him to question the value of the wasp in a biological control program

against mole crickets. In the late 1980s a different, perhaps more cold-tolerant strain of *L. bicolor* was imported from Bolivia, released in northern Florida (approximately 29°40'N), and a decade later appeared not only to be established but also to be spreading (Frank and Parkman 1999), indicating at least that mole crickets in some unknown proportions were being parasitized. Again, no follow-up evaluation has ever been made, either of parasitism efficiency or of geographical spread of the wasp (Frank and Parkman 1999).

Noting the lack of data on percent parasitism, at the same time that the wasp is being touted as a major player in integrated pest management of mole crickets in the southeast (Frank and Parkman 1999), an objective was set to document percent parasitism of pest mole crickets by *Larra bicolor* in the field. A second objective was to analyze for an effect of mole cricket size on percent parasitism.

Materials and methods

In the summer of 1999, 3 pitfall traps were established at each of 2 sites: the University of Florida Horse Teaching Unit (HTU), and the Department of Fisheries and Aquatic Sciences (FAS) field laboratory situated ≈10 km almost directly north of the HTU, in northwest Gainesville. A row of 25 *S. verticillata* plants had earlier been established at each of the sites. Each trap, a modification of the design of Lawrence (1982), consisted of a 20-liter plastic bucket in which four 7.5-cm holes had been drilled every 90° around the top edge; four 7.5-cm dia. PVC pipes, each 3 meters long and with a 2.5-cm slit cut from their entire length, were fitted into the holes.

Traps were checked weekly from 2 September to 30 December 1999 and from 14 September to 7 December 2000, periods of 18 and 12 weeks respectively, and the

samples processed as described above. For the 2000 samples, only mole crickets trapped through 30 November were considered in analysis because an early hard freeze (air temperature = 4°C) marked the end of the wasp season; *Spermacoce* plants were killed at that time and wasps were observed dead on the ground.

Pronotal measurements were grouped into size classes of 0.5- and 1.0-mm intervals for statistical analysis. Proportions of mole crickets parasitized were compared for significant differences between the 2 sites, among total mole crickets trapped per location per year, among size classes of nymphs and between nymphs and adults, and between the 2 years of the study using chi-square tests (Snedecor 1946). Only those samples that included at least 1 parasitized mole cricket, regardless of stage, were used for comparison of total mole crickets against percent parasitized, and only size classes with more than10 individuals were included in analysis of that parameter, to conform to chi-square requirements. Analysis of the significance of mole cricket stage considered only samples that included both nymphs and adults; samples lacking one or the other life stage were excluded.

The success of the wasp in parasitizing the different mole cricket sizes explained before, was evaluated by tracking the developmental success of the parasitic stages collected from pitfall trap captures, and compared with success of parasitic stages in laboratory trials. The success of the parasitic stages was defined as the parasitic larva reaching the pupal stage, which implies the wasp grub kills the host by devouring it and then building a cocoon. Another way to evaluate the wasp's success was by comparing the proportion of wasps reaching the adult stage (adult emergence). Other parameters

describing the wasp's success were the development time, sex ratio, and the possible interaction between molting nymphs and parasitoid success in killing the host.

The proportions of each the three cohorts were compared by the chi-square test and development times were compared by the t-test (Snedecor 1946). The three cohorts evaluated are described as follow: a) Field-parasitized hosts. 40 (nymphs and adults) mole crickets bearing a wasp parasitic stage. These hosts were parasitized by wasps in the field. The parasitized hosts were captured in pitfall traps (described above). The age of the stages (eggs and larvae) attached to mole crickets was unknown. b) Laboratory-induced parasitism (Lab.1). A cohort of 32 nymphal mole cricket hosts (in which molting had occurred sometime in the five days before the test) was selected. The hosts were exposed to laboratory-reared wasps. Parasitized hosts were confined as in the field cohort. c) Laboratory-induced parasitism (Lab. 2) A cohort of 37 (close to molt) nymphal mole cricket hosts was used. Their last molt was at least 15 days before the test.

The reason for use of the two laboratory cohorts was based upon previous experiences rearing mole crickets, where molting of medium to large nymphs occurred every 15 to 30 days. In this way the first laboratory cohort did not face the risk that the parasitoid would be shed along with the exuviae. The developing parasitoids in the second laboratory cohort had a high chance of encountering molting by the host. This second laboratory cohort would provide a good insight of the wasp's success while developing on molting nymphal host stages.

The three cohorts were kept under the same laboratory conditions, 26 ±0.5°C, 14:10 (D:L) photoperiod, 60% RH. The containers were filled with autoclaved sand, kept wet by adding tap water once a week. While alive, the hosts were fed twice a week with

Purina cricket chow. The transparent walls of the containers allowed assessment of parasitoid development, pupation, and wasp emergence.

The differences in sizes between sexes of *Larra bicolor* were evaluated from the pupae and adults obtained from those field-parasitized mole crickets captured from pitfall traps. Pupae were measured alive, or their exuviae were measured. Adults were marked with paint at emergence, to record association with the pupae from which they had emerged, and were measured after death. The variables considered were adult wasp: body length, left forewing span, and thoracic width; pupa: length, and maximum width (of the thorax). All variables were measured in millimeters. The variables were sorted by sex and analyzed independently, and by coupling corresponding pupal with adult dimensions. The statistical analyses were linear regression, t-test comparison, and 99% confidence intervals of the mean per variable between sexes.

Results

Percent parasitism

In total, 195 mole crickets were trapped at HTU during the 2 seasons of the study, with a total parasitism of 24.1%. At FAS, 204 mole crickets were trapped during the 2 seasons; 18.1% of these were parasitized (Table 48, Figure 35). There was a significant difference ($p < 0.10$) in the percent parasitism of mole crickets trapped between the 2 sites in 1999, with 22.3% (n = 31 of 139 individuals parasitized) at HTU versus 12.6% (n = 11 of 87 parasitized) at FAS, but in 2000 percentages of 28.6 (n = 16 of 56) at HTU and 22.2 (n = 26 of 117) at FAS were statistically the same.

Table 48. Numbers of *Scapteriscus vicinus* mole crickets, and numbers parasitized by the parasitoid wasp *Larra bicolor*, trapped in pitfall traps at 2 locations during 2 seasons in Gainesville, Florida.

	Horse Teaching Unit (HTU)			Fisheries & Aquatic Sciences (FAS)		
Year	No. mole crickets	Number parasitized	% parasitized	No. mole crickets	Number parasitized	% parasitized
1999	139	31	22.3	87	11	12.6
2000	56	16	28.6	117	26	22.2
Totals	195	47	24.1	204	37	18.1

The parasitism proportion at FAS recorded during the two years was statistically different ($p < 0.10$), because it was almost twice in 2000 that of 1999. No difference was observed between years at HTU. Data for the two years and two locations were pooled before testing for a difference of mole cricket sex on percent parasitism, but no difference was found.

Over the 2 years of the study, pronotal lengths of trapped mole crickets ranged from 5.4 to 9.15 mm (mean = 7.63 mm, n = 211) for nymphs and 6.9 to 9.55 mm (mean = 8.32 mm, n = 187) for adults. The average pronotal length of adult males (mean = 8.42 mm, n = 124) was significantly greater than that of females (mean = 8.10, n = 63; two-sample t-test, $P << 0.01$). The adult data are compatible with ranges of 7.0 to 10.0 (mean = 8.3) mm for adult males and 6.6 to 8.8 (mean = 7.9) mm for adult females reported by Forrest (1987). There was no evidence that larger nymphs (>7.5 mm) were more heavily parasitized at either site in either year, and there was no evidence that adults were parasitized in higher proportions than were nymphs (Fig. 36). Mole crickets were thus grouped together regardless of size or stage for further analysis.

Overall parasitized nymphs were compared with overall parasitized adults. There was a statistical difference during 1999 at both places, but 2000. Pooled data from the two places and the two years showed no difference.

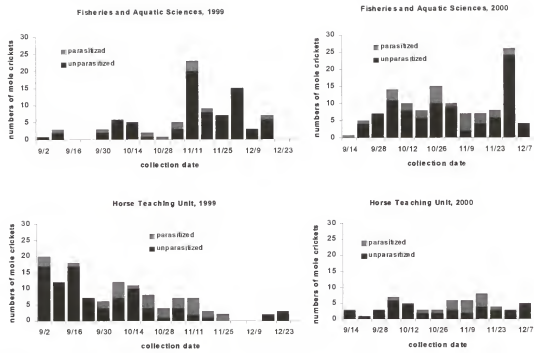

Figure 35. Numbers of parasitized and unparasitized *Scapteriscus vicinus* mole crickets trapped in pitfall traps at 2 locations during 2 seasons in Gainesville, Florida, by date of collection.

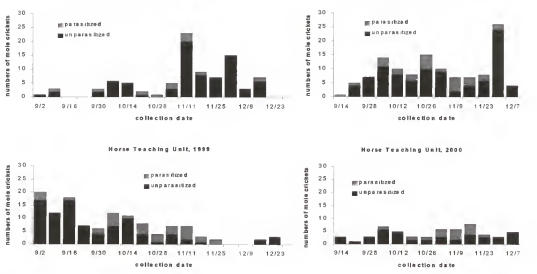

Figure 36. Numbers of nymphs and adults of *Scapteriscus vicinus* mole crickets, and proportions of each parasitized by the parasitoid wasp *Larra bicolor*, trapped in pitfall traps at 2 locations during 2 seasons in Gainesville, Florida, by pronotal size class.

Parasitoid success

There were no differences among the three cohorts in the proportions of wasp success in killing the host, wasp emergence, wasp sex ratio, unrelated host death, and development time overall and by sex (Table 49). In the Lab. 2 cohort, 19% of the developing parasitoids were shed along with the molt of nymphal hosts. All the mole cricket nymphs that molted survived to the adult stage.

Table 49. Success of *Larra bicolor* parasitizing *Scapteriscus vicinus*.

	COHORTS			
SUBJECT	FIELD	LAB. 1	LAB. 2	OVERALL
Parasitic success[1]	93.3 a	100.0 a	100.0 a	97.6
Parasitoid died[2]	6.6	0.0	0.0	2.4
Unrelated host death[3]	25.0 a	15.6 a	13.9 a	18.5
Wasp adult emergence [4]	50.0 a	59.3 a	48.0 a	52.4
Wasp Sex ratio	0.53 : 0.46	0.37 : 0.63	0.5 : 0.5	0.47 : 0.53
Parasitoid shed by host molt[5]	0.0	0.0	19.3	7.3
Parasitoid development (egg to adult) in days.				
Overall	130.0	155.1 a	148.3 a	144.5
Females	132.0	146.1 a	151.8 a	142.2
Males	128.0	162.1 a	144.8 a	146.5

1. Wasps that reached the pupal stage.
2. Proportion not including unrelated host death.
3. Hosts that died for unknown reason, not attributable to development of the parasitoid.
4. Proportion considering all wasp pupae. Not considering parasitoid death nor unrelated host death.
5. Eggs and larvae that were shed with the host exuviae when molting.

Means followed by same letters are not statistically different (Duncan's test). Comparison by subject.

Sex dimorphism

Thirty-four adult wasps emerged out of the total field-parasitized mole crickets captured from pitfall traps, and were 16 males and 18 females. They, and the pupae from which they developed, were measured. The t-test comparing all five variables between wasp sexes gave highly significant differences between all comparisons, indicating a good separation of mean measurements (Table 50). Regressions of pupal dimensions with

corresponding (coupled) adult dimensions indicated no significant linear relationship between any combination per wasp sex nor overall.

Table 50. Sex dimorphism in adult and pupal stages of *Larra bicolor* developed on naturally field-parasitized host mole crickets.

	Adult wasp			Pupal wasp	
Wasp	Thorax[a]	Wing[b]	Body[c]	Length[d]	Width[e]
Female[f]	3.89 ± 0.2 a	12.88 ± 0.6 a	18.55 ± 0.9 a	20.74 ± 1.1 a	8.38 ± 0.4 a
Male[f]	3.31 ± 0.1 b	11.62 ± 0.4 b	14.02 ± 0.6 b	18.01 ± 0.7 b	7.51 ± 0.3 b

a. Wider segment of the pronotum (mm).
b. Left forewing span (mm).
c. Body length from the head to the tip of the abdomen (mm).
d. Maximum length of the unhatched pupa (mm).
e. Maximum equatorial diameter of the pupa (mm).
f. Mean ± 99% CL.
Means followed by different letters between sexes are statistically different; 't' test..

Despite the clear separation of mean measurements, there was overlap in size ranges of the two sexes. The pupal lengths of 37 % of the males overlapped with those of females, and the pupal widths of 68% of the males likewise overlapped (Fig. 37).

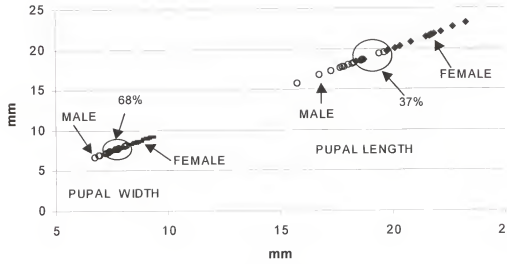

Figure 37. Pupal length and pupal width comparison between sexes of *Larra bicolor*. Circles show the proportion of overlapping sizes between sexes.

Because of overlap, pupal dimensions did not provide a highly reliable means of differentiating sex of adults. When a wasp emerges, a portion of the abdominal apex of the pupal exuviae is lost, making it impossible subsequently to measure the length.

Discussion

The true mortality of mole crickets subjected to parasitism by *L. bicolor* can be shown to be much higher than the average 24% reported for the 13-week period ending 7 December 2000 at both sites. Development time of *L. bicolor*, from egg through larval stages, is three weeks (Castner 1983), and there is a continuous overlapping of parasitoid generations. However, *Scapteriscus vicinus* is univoltine (Walker 1985b). Consider the following analogy: if a regiment of soldiers is subject to 24% casualties during any 3-week period of a war, what will be the total percentage attrition of the regiment after 13 weeks assuming no reinforcements? The answer is about 70% for the soldiers as for *S.*

vicinus, calculated as 100 $(1-(1-0.24)^{4.33})$ where 4.33 is 13/3. This high level of mortality nevertheless excludes mortality occurring in other months.

Juvenile stages of *S. vicinus* occur in north Florida from early May until December, reaching their maximum numbers during July and August. Data on the number of nymphal stadia are inconclusive at present; Walker (1985b) reported 6 to 7, Braman (1993) reported 10. Adults start appearing in late August, reaching their maximum numbers during late November and December. This species overwinters as large nymphs (15%) and adults (85%) (Hayslip 1943, Walker 1985b).

Limiting parasitism only to adult mole crickets would provide the wasp a narrow window of opportunity for success; wasps would have largely died out at the time of adult population peak. Parasitizing nymphs as well as adults enable the wasp to increase generation turnover and population numbers, but with risks for wasp life cycle success. Although 81% of eggs and larvae were observed to reach the pupal stage (so killing the host) while developing on ready-to-molt hosts (Lab. 2 cohort), 19% did not succeed. In at least four cases the parasitic larvae were able to remain on the host after it molted, and in one occasion a medium-large wasp larva was shed along with the host exuviae. The high wasp success, 93 to100% (97% overall), in reaching the pupal stage indicates the very high efficiency of this parasitoid in killing the host, and the 48 to 59.3 (52.4% overall) of wasp emergence supports it. Increased percent parasitism during the second year at one of the study sites (FAS) suggests that *L. bicolor* increased its population there.

It was believed that small or medium host nymphs might be limited in their capacity to provide enough nutrients for wasp grub development; the nymphs' own development is further limited by the presence of the grub (Castner 1988). Also, small

nymphs might be more vulnerable to predation, causing the loss of any attached eggs or grubs. However, this work shows not only that nymphs as small as 6.5 mm pronotal length were parasitized in the field, but that development of wasp grubs occurred successfully on such small hosts.

No difference was seen between development time of males and of females (Table 24). This does not match Castner's (1988) observations; he considered that males develop in a shorter period of time than do the females. Half (≈50%) of the wasps that reached the pupal stage emerged in a period of 152.2 d. The remaining half did not emerge and died, which can be attributed to inadequate laboratory conditions to keep them alive, or to the lack of a necessary environmental stimulus to emerge.

One of the most difficult problems in studying *Larra bicolor* development is the prolonged time for the pupa to develop, causing delay and inaccuracy in knowing the wasp's sex, one of the most important items in evaluating the parasitoid's performance as a biological control agent. In order to solve this problem, the wasp sexes were characterized by their dimensions. The pupal length and width may be used as a method of determining the adult's sex, but this is not entirely reliable because of overlap in size ranges. The body length of the adult correlates well with its sex, but is less accurate than dissection of the genitalia. The problem to wait such a long time for the adult to emerge and then know its sex can be solved by taking the pupal dimensions (length or width) that can predict the wasp sex.

APPENDIX C
SOIL TEMPERATURE ESTIMATE

Problem description

During developmental studies, the two parasitoids of mole crickets, the tachinid fly *Ormia depleta* and the digger wasp *Larra bicolor*, were kept underground at field temperatures in the natural habitat of their host. The soil temperatures were constantly recorded every 12 min at a range of soil depths. During 1998, I used two dataloggers that stopped working very often. In June 2000 a digital datalogger was purchased, which has four thermocouples to record the same number of temperatures at the same time. Unfortunately one thermocouple did not work and the datalogger recorded temperature from three points, two underground and one from air. The soil depths where temperature was recorded were 7.5 and 22.5 cm, and the air temperature was recorded at 1.8 m. Recordings were made every 30 min. In October of the same year, a fourth thermocouple was purchased along with another datalogger with the capability to record temperature from one point only. The four-point recording temperature datalogger was set to record temperature at four soil depths, 7.5, 22.5, 37.5, and 52.5 cm respectively. The single thermocouple datalogger was set to record air temperature at 1.8 m. Both dataloggers were set to record temperature every 30 min.

The only problem with the four thermocouple datalogger was that it stopped working twice due to a loose battery connection. The dates when temperatures were not recorded are from 16-23 August, from 29 September to 11 October, and 2-4 November.

Thus this problem caused the lack of those temperature recordings during the development of the parasitoids underground. Therefore, it was necessary to estimate soil temperatures from recordings from the meteorological station nearest to the location of the experiments. Fortunately, the Florida Automated Weather Network (FAWN) has a station less than a mile from the experimental plot. The FAWN records soil temperature at 15 cm depth underground and air temperature at 1.8 m, among other conventional recordings. The FAWN records are taken every 15 minutes.

The FAWN soil temperature is recorded at an intermediate depth of my experimental plot, where thermocouples were set at 7.5 and 22.5 cm, which means FAWN was set at the middle depth of my experiment. I compared the FAWN soil records with my own that were recorded at the same time. This provided an intermediate set of records (15 cm) between my uppermost depths (7.5 and 22.5 cm), which I took to be the expected result. The FAWN records followed the trend of the temperature recorded from the experiments, but failed to match the precise values of my data (Figure 38).

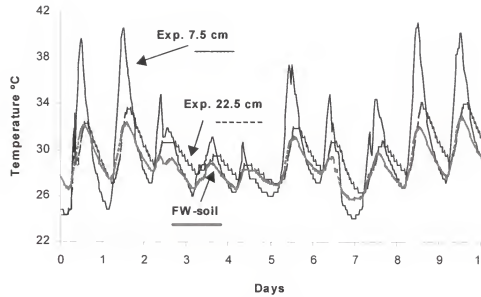

Figure 38. Temperatures recorded during 10 days in July from the soil in the experimental area (Exp.) at two depths (7.5 and 22.5 cm.) and from soil (15 cm depth) recorded by the Florida Automated Weather Network (FAWN).

I built a mathematical model to obtain a better estimate of the temperatures at the study site during the periods when the experimental data were lacking.

Materials and methods

Temperature estimates.

The temperature estimate was performed by applying stepwise linear regression analysis. The independent variable was the temperatures recorded by the Hobo datalogger in the experimental area; the dependent variables were soil and air temperatures from FW. The models evaluated, adding the coefficients in delayed times, are shown in Table 51.

Table 51. Linear equation models proposed to estimate soil temperatures.

Model	Description
Model 1	$Y_{7.5} = a + b1(S_{t0})$
Model 2	$Y_{7.5} = a + b1(S_{t0}) + b2(S_{t-1})$
Model 3	$Y_{7.5} = a + b1(S_{t0}) + b2(S_{t-1}) + b3(S_{t-2})$
Model 4	$Y_{7.5} = a + b1(S_{t0}) + b2(S_{t-1}) + b3(S_{t-2}) + b4(S_{t-3})$
Model 5	$Y_{7.5} = a + b1(S_{t0}) + b2(A_{t0})$
Model 6	$Y_{7.5} = a + b1(S_{t0}) + b2(A_{t0}) + b3(S_{t-1}) + b4(A_{t-1})$
Model 7	$Y_{7.5} = a + b1(S_{t0}) + b2(A_{t0}) + b3(S_{t-1}) + b4(A_{t-1}) + b5(S_{t-2}) + b6(A_{t-2})$
Model 8	$Y_{7.5} = a + b1(S_{t0}) + b2(A_{t0}) + b3(S_{t-1}) + b4(A_{t-1}) + b5(S_{t-2}) + b6(A_{t-2}) + b7(S_{t-3}) + b8(A_{t-3})$

Where:
$Y_{7.5}$ = Temperature recorded at the experiment at 7.5 cm depth underground.
S = Soil temperature from Florida Automated Weather Network (FAWN) database
A = Air temperature from Florida Automated Weather Network (FAWN) database
t_0 = Temperature recorded at time zero
t-1 = Temperature delayed one time step (30 min).
t-2 = Temperature delayed two times step (30 min).
t-3 = Temperature delayed three times step (30 min).

Those models were run including either 500, 1000, or 2000 data entries; each data entry was the temperature recorded each 30 min underground in the experimental area. The linear equation obtained by running the first data set was then run to another data set, a group of data recorded at various dates, not included in the calculation of the equation, so that the equation should mimic the new temperature data set.

The predicted data were plotted with the new temperature data, thus evaluated by two ways, first the predicted values should follow the experiment's data trend, and second the predicted data should match the values of the experiment's new data. Their R^2 values and the significance of their coefficients evaluated the proposed models. The equation that better fit those criteria would be selected to estimate the missed data.

The data set that was used to build and evaluate the models consisted of the data next to the periods where the experimental data were not recorded. Therefore, the equation was built using the data previous to the empty period, and then the validation

was run with data after the same empty period. It was assumed that one equation would be required per soil depth to estimate the missed data of the two different periods.

Results

Before starting to build the temperature model to estimate the missed data, the estimation procedure was first applied to all the known and contiguous series of data. This was intended to build and to validate the model working with known data, which are contiguous in time. To accomplish this trial, the July data set was chosen.

The proposed models calculated include 500, 1000 and 2000 data points. They were plotted along with independent and dependent variables, so that their trends and values were compared. The models including soil temperature as the only independent variable are depicted in Figure 40, where the model 1 (Figure 39, A) appeared to be the most variable, particularly when including 1000 data points; the other three models (Figure 39, B, C, and D) showed very similar predicted outputs when using any of the input (500, 1000, 2000) levels.

The comparison of the regression coefficients of the models considering the soil temperature as the only independent variable is shown in Table 52. The R^2 value of model 1 is clearly lower than that of the other three models. The R^2 values for models 2 to 4 are very similar. The regression coefficients of model 1 are all statistically significant. The coefficients of model 2 were all significant when using 500 and 1000 data points, but one coefficient was not significant with 2000 data points. Models 3 and 4 presented at least one coefficient not significant with the three (500, 1000, 2000) levels of data points, becoming less significant as it included more coefficients.

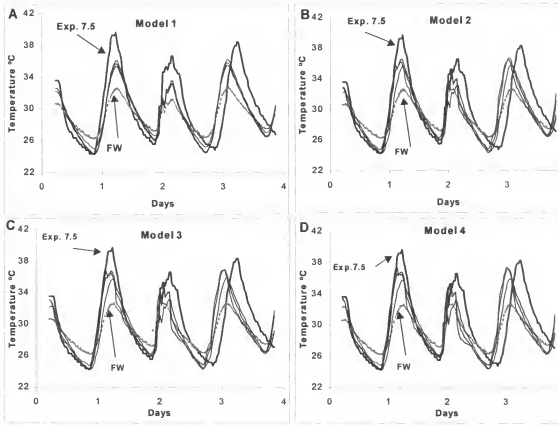

Figure 39. Efficiency of soil temperature models to estimate temperature at 7.5 cm depth. Independent variable: Soil temperature from the experimental area (Exp. 7.5) at 7.5 cm depth. Dependent variable: Soil temperature (FAWN -soil) from the Florida Automated Weather Network.

Table 52. Coefficient of determination values and statistical significance (p-values) of slope and intercept for four models estimating soil temperature, using 500, 1000, or 2000 data points.

	Model 1			Model 2			Model 3			Model 4		
	500	1000	2000	500	1000	2000	500	1000	2000	500	1000	2000
R^2	0.66	0.77	0.81	0.70	0.80	0.81	0.71	0.81	0.81	0.73	0.81	0.81
a	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
b1	0.001	0.001	0.001	0.001	0.001	0.001	> 0.05	0.001	0.05	> 0.05	0.001	0.05
b2				0.001	0.001	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05
b3							0.001	0.001	> 0.05	> 0.05	> 0.05	> 0.05
b4										0.001	0.001	0.05

The temperature estimates by the proposed models including soil an air temperature as the independent variables are depicted in Figure 40. A 4-day output is shown because the lines become difficult to discern visually with longer outputs. The outputs in Figure 40 show how similar the predicted temperatures are among the three levels of input.

Model 1 (Figure 40, A) appeared to be the most variable, particularly when including 1000 data points. The other three models (Figure 40, B, C, and D) showed very similar predicted outputs at the three levels of input.

Comparison of the regression coefficients of the models considering soil and temperature as the independent variables is shown in Table 53. The R^2 value of model 1 is clearly lower than for the other three models. The R^2 values for models 2 to 4 are very similar. The regression coefficients of models 1 and 2 are all statistically significant. Most of the coefficients of models 3 and 4 are not significant for the three data input levels, becoming getting less significant as they include more coefficients.

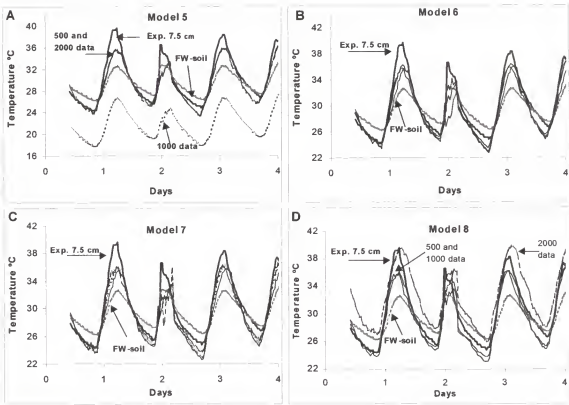

Figure 40. Efficiency of soil temperature models to estimate temperature at 7.5 cm depth. Independent variable: Soil temperature from the experimental area (Exp. 7.5) at 7.5 cm depth. Dependent variable: Soil temperature (FAWN-soil) and Air temperature (not shown) from the Florida Automated Weather Network.

Table 53. Coefficient of determination values and statistical significance (p-values) of intercept and slope of four models estimating soil temperature with soil and air temperature as independent variables, using 500, 1000, or 2000 data points.

	Model 5			Model 6			Model 7			Model 8		
	500	1000	2000	500	1000	2000	500	1000	2000	500	1000	2000
R^2	0.71	0.82	0.84	0.72	0.83	0.86	0.73	0.83	0.87	0.74	0.84	0.88
a	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
b1	0.001	0.001	0.001	0.001	0.001	0.001	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05
b2	0.001	0.001	0.001	0.001	0.05	0.001	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05
b3				0.05	0.05	0.001	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05
b4				0.05	0.001	0.001	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05
b5							> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05
b6							> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05
b7							> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05
b8							> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05

Temperature estimate of missed data

The differences between models 1 and 2 were not quite evident, considering soil alone or with air as independent variables. Regardless of input level (500, 1000, or 2000 data points), the predicted temperatures were very similar (except model 1 with 100 data with soil and air variables). The more apparent differences were the R^2 values that were higher in models 2 and 6 than in models 1 and 5, with all coefficients statistically significant. Models 3 and 4 models 7 and 8, although producing similar predicted outputs, showed no significance among their coefficients, with mostly similar R^2 values compared with models 2 and 6.

The choice of the best model to estimate soil temperature lay between models 2 and 6, with 500 or 1000 data points. Considering the higher R^2 values, the statistical significance of all coefficients and the not very complicated data management required to achieve a good temperature prediction, model 2 was chosen, with 1000 data points. Then this model was applied to estimate the temperatures during those periods when data from the experimental area were lacking.

The model building was performed using the temperatures recorded in the experimental area at 7.5 cm depth from 5 July to 16 August 2000. The data are shown in Figure 42, where is evident the disparity between the temperature in the experimental area at 7.5 cm depth and the FW recordings. The similarity of temperatures at 22.5 cm depth and those from FW are evident in the same Figure 41.

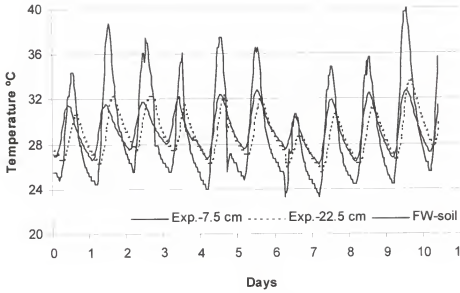

Figure 41. Temperatures recorded at two depths from the experimental area (Exp.) and from the alternative temperature record source (FAWN). Period from 5 July – 16 Aug. 2000.

By applying model 6 with 1000 data points, the proposed model was:

$Y = -1.96286(S_{t0}) - 0.07562(A_{t0}) + 3.113706(S_{t-1}) + 0.546506(A_{t-1}) - 16.8321$. Thus, applying this model to the next data series, including dates from 23-30 August 2000 the predicted temperatures, shown in Figure 42, fitted the observed temperatures in trend and values.

The similarity of the FW-soil data to the temperature records of the experimental area was evaluated with the data after and before the missed data, and shown in Figure 43 (A and B respectively).

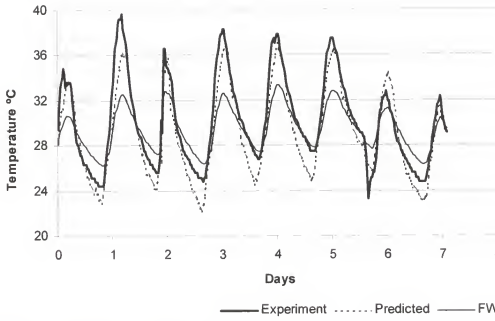

Figure 42. Temperatures recorded at 7.5 cm depth from the experimental area and from the alternative temperature record source (FAWN). Period from 23-30 Aug. 2000. The predicted temperature was estimated with the equation: $Y = -1.96286(S_{t0}) - 0.07562(A_{t0}) + 3.113706(S_{t-1}) + 0.546506(A_{t-1}) - 16.8321$.

The same equation applied to the data of August (Figure 43) was applied to the data next to missed data of September. The output showed the same trend but failed to match the values, so that it was decided to build a model using the more contiguous data to the missed ones, following the same procedure explained before. The data before the missed period are shown in Figure 44, where it is evident the lower seasonal temperatures, noting fewer differences between the experimental and FAWN temperatures. Thus the model building procedure was applied, producing the equation: $Y = 6.61423(S_{t0}) - 0.4854(A_{t0}) - 4.4884(S_{t-1}) + 0.35548(A_{t-1}) - 20.061$. This equation was then run with the observed temperatures after the missed period, with results in Figure 45. The

predicted temperatures followed the trend and matched the values of the observed temperatures in the experimental area.

Similarity of the FAWN soil temperatures to those observed in the experimental area at 22.5 cm depth was proved by plotting both temperatures using data before and after the missed period (Figure 46, A and B respectively).

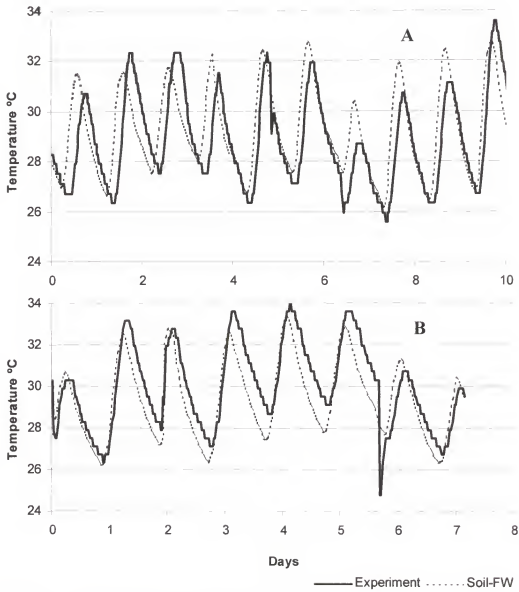

Figure 43. Temperature for 22.5 cm depth underground before and after the period of 16-23 August 2000. A) Temperatures recorded before the missed temperature period. B) Temperatures recorded after the missed temperature period.

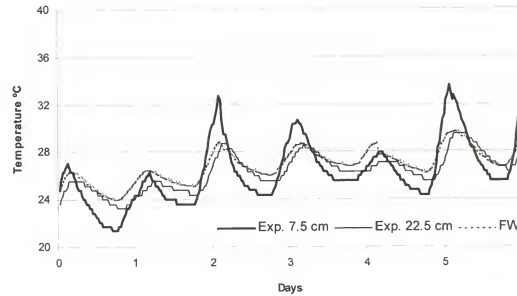

Figure 44. Temperatures recorded at two depths from the experimental area (Exp.) and from the alternative temperature record source (FAWN). Period from 29 Sep. – 11 Oct. 2000.

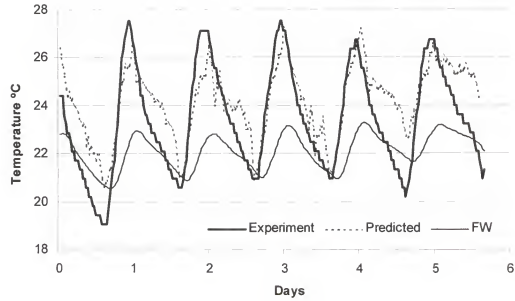

Figure 45. Temperatures recorded at 7.5 cm depth from the experimental area and from the alternative temperature record source (FAWN). Period from 29 Sep. - 11 Oct. 2000. The predicted temperature was estimated with the equation: $Y= 6.61423(S_{t0})-0.4854(A_{t0}) -4.4884(S_{t-1}) +0.35548(A_{t-1})-20.061$.

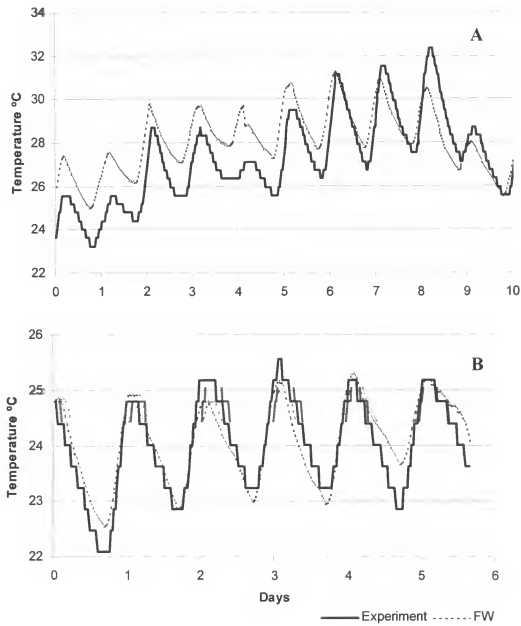

Figure 46. Temperature model for 22.5 cm depth underground for the period of 29 Sep. – 11 Oct. 2000. A) Temperatures recorded before the missed temperature period and used to build the model. B) Temperatures recorded after the missed temperature.

Discussion

The datalogger set to record soil temperatures in the experimental area stopped working twice, missing the temperatures of several days. This problem coincided with phenological studies of two parasitoids that develop mostly underground. An alternative temperature data set was considered to supply the missing temperatures. The most proximate climate station was less than a mile from the experimental area; the Florida Automated Weather Network (FAWN) has an automated climate datalogger that includes soil temperature recordings at 15 cm depth. The datalogger in the experimental area, however, was set to record temperatures at 7.5 and 22.5 cm depth. A comparison of the records was made, and it showed disparity between data from 7.5 cm depth in the experimental area and those from FW (soil). Differences between data from 22.5 cm depth in the experimental area and those from FW (soil) were negligible.

Due to lack of fit between the upper sub-chamber temperatures and those from FAWN, a model was developed to estimate the missed temperatures at the experimental area. The linear equation model developed to estimate temperature from August was applied to estimate temperatures from October, noting a poor fit of the predicted data. The same result occurred when using the model based upon October data to predict August temperatures.

The lack of fit of the models built based upon data from different seasons of the year led to building and use of models based upon data of the same season. The resultant predicted data matched the temperatures used for validation (data after periods when temperatures were missed).

The following speculation seems appropriate:

i. Perhaps mole crickets tried to dig deeper away from soil surface to prevent desiccation. The upper chamber (sub-chamber A= 0-15cm depth) lost moisture at higher rate than deeper sub-chambers, particularly the first 5 cm.

ii. 95% of the hosts died at the bottom of the sub-chambers –during 1999 the chambers consisted of PVC pipes up to 60 cm length kept underground without divisions, where parasitized hosts died at the bottom.

iii. It is very likely the development of the parasitoids occurs deep underground (based upon i and ii) –deeper than the upper sub-chamber- where temperature (and moisture) is more stable, escaping the higher temperatures, particularly during the summer months (see Tables 46 and 47).

iv. That soil temperatures at depths of 22.5 cm or deeper were so similar in the experimental area and at FAWN, suggests that low variation can be expected in Alachua County, and probably elsewhere in north Florida.

v. If the assumption made (in iv) is true, then the proposed models in chapters 3 and 4 can be applicable to a wider area to predict development of the wasp *Larra bicolor* (short development during spring and fall), and the fly *Ormia depleta.*

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BIOGRAPHICAL SKETCH

Hector Cabrera-Mireles was born on July 25, 1957, in Monterrey, Nuevo Leon, México. He grew up in Monterrey, where he attended school and graduated from high school in 1973. He then went to earn his Bachelor of Science degree in parasitology from the agronomy school at the Universidad Autónoma de Nuevo León (UANL). After graduation, he went to work for INIFAP, in Tabasco State, southern México. He worked there on coconut palm pests. After five years, he was accepted into the graduate program of the Instituto Tecnológico y de Estudios Superiores de Monterrey (ITESM), to study plant health. After finishing his master's degree he continued working for INIA (today INIFAP) in the research program on fruit flies research, focusing on mango fruits. In 1997 he was accepted into the graduate program of the University of Florida Entomology and Nematology Department to study biocontrol. After finishing his doctoral degree, he will continue working for INIFAP in Veracruz state, México.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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